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Fetal Alcohol and Adolescent Behavior: The Effects of Postnatal Binge Ethanol Exposure on the Behavioral Development of Adolescent Animals

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FETAL ALCOHOL AND ADOLESCENT BEHAVIOR

The Effects of Postnatal Binge Ethanol Exposure on the Behavioral Development of Adolescent Animals

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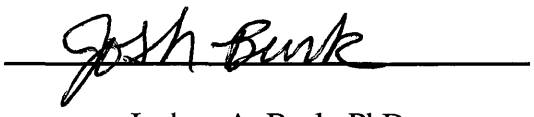

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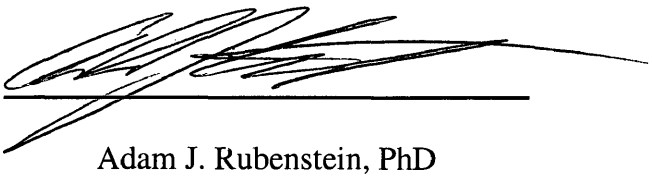

Adam J. Rubenstein, PhD

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ABSTRACT

The purpose of this study was to further develop a postnatal model of the cognitive and behavioral deficits typically associated with adolescents prenatally exposed to alcohol. The effects of postnatal binge-like ethanol exposure on the adolescent rat were examined. Intragastric intubations of ethanol (5.25g/kg/day) were administered to male and female Sprague-Dawley rats on postnatal days (PD) 4-9, a period thought to be equivalent to the brain growth spurt present in human third trimester. This ethanol exposure caused (a) disruptions in the rate of response habituation spanning from the pre-weanling period to adolescence, (b) altered adolescent social competencies, (c) and increased preference for ethanol ingestion during adolescence. Implications pertaining to the duration of alcohol-related cognitive and behavioral dysfunctions as well as the efficacy of the postnatal intubation model of fetal alcohol effects are discussed.

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Human Fetal Alcohol Exposure

In the western world, fetal alcohol exposure is the single most prevalent non-genetic cause of intellectual impairment in children (Clarren, 1986; Spohr, Williams, & Steinhausen, 1993). Fetal alcohol syndrome (FAS) is a grouping of 3 characteristic symptoms resulting from maternal consumption of large quantities of alcohol during pregnancy: pre- and postnatal growth retardation, facial dysmorphology, and several central nervous system (CNS) dysfunctions (Jones & Smith, 1973). Cognitive dysfunctions include low IQ, impaired short-term memory (STM), poor spatial abilities, and attention deficits; the severity of which varies as a function of timing and dose of the alcohol exposure (Clarren & Smith, 1978).

Ebrahim, Luman, Floyd, Murphy, Bennett, and Boyle (1998) recently found that although alcohol use in the general population decreased between 1988 and 1992 (from 22.5 to 9.5 percent) by 1995 it had increased to 15.3 percent. In addition, Ebrahim, Diekman, Floyd, and Decoufle (1999) showed that binge drinking (defined as five or more drinks per occasion) among pregnant women, a particularly hazardous drinking pattern in terms of alcohol-related risk, increased significantly between 1991 and 1995 (from 0.7 percent to 2.9 percent of pregnant women). This increase in drinking behavior during gestation is reflected in the increasing incidence of

FAS/FAE from 1 in 10,000 live births in 1985 to approximately 1 in 500 live births in 2001 (May & Gossage, 2001).

The diagnosis of FAS identifies only a relatively small proportion of children affected by prenatal alcohol exposure (Connor & Streissguth, 1996). Indeed, in a recent review, May and Gossage (2001) estimated that the prevalence of FAS in the United States is between .05 and 2 per 1,000 live births. However, the overall incidence of clinically documented behavioral and anatomical alterations associated with alcohol-exposure during gestation is estimated to be higher, at least 10 per 1,000 (1 %) of all live births. These diagnoses include not only FAS, but Fetal Alcohol Effects (less severe forms of FAS) such as Alcohol-Related Birth Defects (ARBD) and Alcohol-Related Neurodevelopmental Disorders (ARND).

The term "Fetal Alcohol Effects" (FAE) has been used since the mid 1970's to describe patterns of birth defects following significant prenatal alcohol exposure that do not include all of the facial features or growth retardation seen in FAS (Clarren and Smith, 1978). Even without full-blown FAS, children with known exposure to alcohol demonstrate several learning and behavioral deficits such as shortened attention span and hyperactivity. Both FAS and FAE individuals demonstrate deficits in relatively simple learning tasks and situations that demand attentional focusing (Riley, 1990). Stratton, Howe, and Battaglia (1996) introduced a new classification of prenatal alcohol exposure to replace the vague terminology of FAE. Alcohol-related neurodevelopmental disorder (ARND), refers to damage due to significant prenatal alcohol exposure, but focuses specifically on brain dysfunction (Institute of Medicine, 1996). ARND, unlike FAS, is diagnosed in the absence of facial or other

physical abnormalities. Although patients with FAS have more physical deformities, the brain dysfunctions of people with ARND are often as severe as- if not worse than- brain dysfunctions in patients with FAS (Connor and Streissguth, 1996).

Attention deficit, a hallmark sign of FAS/FAE, appears to be one of the few permanent repercussions of early exposure to alcohol. Indeed, significant disruptions in processes of attention have been noted as early as the first day of life and as late as adulthood (Streissguth, Barr, & Martin, 1983). The following review of the lifespan behavioral manifestations of attention deficits associated with early alcohol exposure will provide the framework for Experiment 1.

Experiment 1. The Duration of Alcohol-Related Deficits in Attention and Nonassociative Learning: From Infancy to Adolescence

Alcohol-Related Attention Deficits in Humans: A Lifespan Analysis

Newborn and older infants with known exposure to alcohol are typically described as irritable, jittery, tremulous, and difficult to feed (Pierog, Chanadavasu, & Wexler, 1979). Indeed, infant studies reveal that diagnosis with FAS/FAE is associated with tremors, a weak suckle, and difficulty "tuning out" redundant sensory stimulation (Connor and Streissguth, 1996).

Previous research suggests that poor habituation to sensory stimuli early in life is predictive of later CNS dysfunction (Lewis, 1969). Habituation is defined as a decrease in the magnitude of a response to redundant or repetitive sensory stimulation. It is considered to be both a fundamental aspect of attention and nonassociative learning, as it involves being able to recognize that a stimulus has previously been encountered and that the stimulus is ecologically non-significant to survival and can be "tuned out". Habituation is therefore essential to more complex forms of attention as it filters out extraneous stimulation and allows for focus and vigilance on a task, which are, in turn, paramount for effective learning. In addition, the habituation paradigm is valuable to the study of attention, especially infant attention, because it requires a minimal motor and cognitive repertoire. Habituation can be measured by recording orienting responses (such as looking time, or cardiac

changes) to a repeated stimulus (Lewis et al., 1967) or by measuring how arousing a repeated stimulus is to a sleeping infant (Streissguth et al., 1983).

Streissguth, Barr, and Martin (1983) measured the effects of fetal alcohol exposure on newborn infants by recording changes in habituation of responses to simple visual and auditory stimulation. In order to minimize the role of the experience on the measurements, Streissguth and colleagues tested infants as soon as possible after birth, leading to a mean participant age of 27 hours. The Brazelton Neonatal Assessment Scale (Brazelton, 1973) was used, as it had successfully differentiated the behavior of newborns prenatally exposed to other drugs (Streissguth et al., 1983). Factor analysis revealed that among the 27 items of the Brazelton scale, 6 independent factors emerged. These included: orientation, reactivity/irritability, habituation, tremulousness/motor immaturity, low arousal, and activity/ muscle tone. The habituation procedure involved presenting a sleeping infant with several sensory stimuli. The infant was first exposed to a visual stimulus (a flashlight beam), which was followed by two auditory stimuli (a rattle, then a bell). The dependent variable was the magnitude of motor response following each of the ten trials of the repeated stimulus presentation. Response habituation scores in this situation were noted as the trial number at which the infant failed to make any further response to the redundant stimulus. Poor habituation was noted when infants showed the lack of a response only after many trials, if at all.

Streissguth et al. (1983) showed that alcohol exposure did not affect sensory orientation responses (OR). Interestingly, infants with known prenatal exposure to alcohol were significantly affected on measurements of habituation, specifically to

the visual stimulus (habituation light) and first auditory stimulus (habituation rattle). These results indicate that detection of the stimulus was intact, but that habituation, which spans attention and nonassociative memory, was severely disrupted. This trend and its implications will be discussed in greater detail pertaining to Experiment 1.

In a comprehensive assessment of the neuropsychological consequences of fetal alcohol effects on school-aged children, Korkman, Autti-Ramo, Koivulehto, and Granstrom, (1998) discussed several deficits found in children ages 5 to 9. By using the NEPSY (developed from "neuro" and "psychology"), a neuropsychological investigation scale for children Korkman and colleagues tested children on measures of attention, language, sensorimotor functions, visual spatial functions, memory and learning. Interestingly, the measures that demonstrated the strongest alcohol-related deficits were those of attention, specifically sustained attention.

Attention deficits due to early alcohol exposure have been consistently reported in school-aged children, so much so, that they are often mistaken for symptoms of Attention Deficit Disorder (ADD) and Attention Deficit Hyperactivity Disorder (ADHD). Two recent studies describe the form and magnitude of the attentional disruptions associated with prenatal alcohol exposure, but each discusses different perspectives on the relationship between alcohol-induced attentional changes and ADD/ADHD.

ADHD is often described as a central feature of the behavioral disruption due to prenatal alcohol exposure in children with FAS/FAE (Coles, 2001). Indeed, children with FAS/FAE are frequently described as hyperactive, distractible, and

having short attention spans (Streissguth, Martin, and Barr, 1984). Tellingly, symptoms of hyperactivity and deficits in learning and attention were the most frequently reported behavioral problems in children with known alcohol exposure (Streissguth et al., 1984). Even pre-school aged children of social drinkers, who do not qualify for the diagnoses of FAS/FAE, often display behavior similar to children diagnosed with ADD/ADHD (Nanson & Hiscock, 1990). Thus, it is possible that the two diagnoses are not mutually exclusive.

Nanson and Hiscock (1990) examined the activity level and attention of a group of children (ages 5-12) prenatally exposed to alcohol and diagnosed with FAS/FAE, as well as a sample of children with ADD/ADHD unrelated to alcohol exposure, and healthy IQ-matched control children, to investigate the relationship between FAS/FAE and ADD/ADHD while controlling for mental retardation. By analyzing parental ratings of attention, intelligence tests, and laboratory based attention-demanding tasks, Nanson and Hiscock determined that children with FAS/FAE, although more intellectually impaired than children with ADD, exhibited attentional deficits similar to children diagnosed with attention deficit disorders. Specifically, they reported that both ADD and FAS/FAE children had difficulty with the investment, maintenance, and organization of attention over time (Nanson & Hiscock, 1990).

In contrast, the work of Coles Platzman, and Raskind-Hood (1997) demonstrated that on neuropsychological measures, the attention deficits associated with children with FAS/FAE were qualitatively different than those deficits present in children with ADD. Coles used Mirsky's Neuropsychological Model of Attention

(Mirsky, Anthony, Duncan, Ahearn, and Kellam, 1991) to differentiate alcohol effects among several types of attention.

Mirsky describes attention as a construct comprised of four unique facets. The first is *focused* attention, and it is defined as the ability to selectively attend to appropriate information. *Sustained* attention is described as the ability to maintain a focused alertness in perceiving a stimulus. *Shifting* attention is the ability to redistribute attentional resources from one stimulus to another, when appropriate. Finally, *encoding* attention is the ability to maintain information in working memory while performing some cognitive process using that information (Mirsky et al., 1991).

By studying these four facets of attention and their relationship to both FAS/FAE and ADD, Coles found that the manifestation patterns of the attention deficits associated with the two groups were unique from one another. The FAS/FAE and ADHD groups had similar scores on intelligence tests, contrary to the trend reported by Nanson and Hiscock (1990). Based on parent and teacher reports, the ADHD children were rated as impulsive and having significant behavioral problems, but the FAS/FAE children were rated as neither. The children in the ADHD group had difficulty in focusing and sustaining attention, whereas the FAS/FAE group had difficulty in shifting and encoding attention, again, converse to the results reported by Nanson and Hiscock. These results challenge the assumption that attentional disturbances seen in children with ADHD and FAS/FAE stem from the same neurocognitive deficits (Coles, 2001). Although the argument of which types of attention are targeted and damaged by prenatal alcohol exposure is not yet resolved,

the presence and impact of attentional disturbances in children with FAS/FAE is evident from the works cited.

Korkman et al. (1998) discussed the relative contribution of the timing of the prenatal alcohol exposure to neuropsychological impairment. Children who were exposed to alcohol during the first trimester only did not demonstrate any altered neuropsychological functioning. For children who were exposed during the first two trimesters, naming was the only significant measure affected by alcohol. However, children exposed to alcohol throughout all three trimesters of pregnancy displayed impairments on almost every measure, specifically naming, receptive language, visuo-motor skills, and attention. What could cause this dramatic differentiation in CNS functioning?

First, the cumulative duration of exposure could be the causal mechanism, which would explain why the longer the exposure, the greater the number and magnitude of deficits. One way to confirm this hypothesis would be to study children who were also exposed for two trimesters, but possibly the second and third. On the other hand, the rise in behavioral deficit due to exposure throughout pregnancy, as opposed to the first two trimesters, could be due to the sole, robust contribution of exposure during the third trimester.

During this period, the brain goes through a period of immense synaptogenesis and neuronal growth, with particular emphasis on cortical migration and cellular connections (Dobbing & Smart, 1974). If a fetus is selectively exposed to alcohol during this critical period, known as the "brain growth spurt", would the neuropsychological deficits be as robust as those observed after exposure throughout

pregnancy? One way to investigate this question would be to develop an animal model to mimic third trimester alcohol exposure, a period that, in rats, occurs postnatally, during the first two weeks life.

Developing Animal Models of Human Fetal Alcohol Exposure

Animal, especially rodent, models are valuable for exploring the underlying mechanisms and behavioral effects in the study of alcohol as a teratogen (Riley & Barron, 1989; Hannigan, 1996). A teratogen is defined as any agent with the ability to cause atypical or abnormal development of a fetus (Kopera-Frye, Carmichael-Olson, & Streissguth, 1997). Currently, no comprehensive animal model for FAS exists, as the offspring of pregnant animals administered alcohol do not yet display all the clinical signs observed in humans (Hannigan, 1996). However, animal models of early alcohol exposure such as FAS and FAE have strengthened and advanced our insight into the risk factors, pathology, and biological mechanisms of early alcohol exposure (Hannigan, 1996; Riley & Barron, 1989; Driscoll, Streissguth, & Riley, 1990). In addition, rodent models are essential to the development of therapies to counter the behavioral dysfunctions linked to early exposure to alcohol (Riley & Barron, 1989). Work has begun studying the ameliorative effects of handling, enriched environments, and pharmacological interventions of the behavioral deficits associated with perinatal ethanol exposure (Hannigan & Berman, 2000).

Although the pattern of CNS development across species is similar, there is great interspecies variability in the timing in which birth occurs in relation to the development of the CNS (Dobbing & Smart, 1974). In order to generalize an animal model evaluating the effects of ethanol insult back to humans, the alcohol exposure

must be based on brain growth periods comparable to those in humans (Dobbing & Smart, 1974). In rats, early postnatal life coincides with a period of rapid CNS growth (known as the brain growth spurt) that occurs in humans during the third trimester of gestational development (Riley & Barron, 1989; Melcer et al., 1994). Thus, one general limitation of exposing rats to alcohol prenatally is that the third trimester-targeted period of brain growth does not occur until after the animal is born, specifically during postnatal days (PD) 4-9.

Because naive rats of this age will not preferentially drink alcohol, to model fetal alcohol exposure, the route of administration must occur by more invasive means. Several techniques have been used to administer ethanol to animals postnatally, each with advantages and disadvantages. The first method is injection of an alcohol solution directly into the peritoneum. This method produces rapid intoxication and is a relatively quick procedure, however, the repeated injections can be stressful for the animal and this route of administration is not very naturalistic compared to exposure in utero. When a developing fetus is exposed to alcohol, it is through the placental interface after the mother has ingested alcohol, not by a direct injection to the fetus' system.

Another method is an artificial rearing technique known as the pup-in-a-cup method (Hall, 1973). This involves intragastric cannulation, the implantation of a gastric fistula containing a tube (attached to an infusion pump to deliver fluid) into the stomach of an animal. Advantages of this method of alcohol administration are that the implantation only occurs once; minimizing stress on the animal, and alcohol is delivered directly to the stomach, where natural digestive processes can commence.

This method also offers strict control over how much fluid and nutrients the subject ingests, a factor that may confound alcohol effects with less stringent administration methodologies. However, this method is also stressful because after cannulation, the pup must be individually housed in an artificial environment (a Styrofoam cup floating in a water bath) and is not afforded the benefits of early social interactions with the dam and littermates, which, during critical periods of brain growth, may be a significant factor in the development of these animals (Diaz, 1991).

A third option, the present methodology, is intragastric intubations. This method of alcohol administration, as developed by Serbus, Young, and Light (1986) involves advancing a small, flexible tube through the mouth, down the esophagus, and into the stomach. This procedure is quick, usually lasting less than one minute, and compared to artificial rearing, produces healthier and heavier pups with more consistent blood alcohol levels (BAL) (Lillquist, Highfield, and Amsel, 1999). In addition, the metabolism of alcohol occurs normally and between administrations the pups are returned to the home cage to reap the benefits of interaction with the dam and littermates. Although the procedure must occur repeatedly to maintain the desired BAL, the intubations are less stressful for the animal than is artificial rearing (Lillquist et al, 1999).

Animal Models of Alcohol-Induced Habituation Deficits: A Developing Lifespan Analysis

One of the most devastating and obvious long-term effects of prenatal alcohol exposure is its impact on the developing nervous system. Two recent studies have

explored the attention deficits associated with heavy fetal alcohol exposure by investigating ethanol's effects on response habituation in young rats.

Hayne, Hess and Campbell (1992) explored the effects of prenatal ethanol exposure on attention in the rat. By using the changes in heart rate to an olfactory stimulus as the dependent measure, Hayne et al. found that prenatal alcohol exposure does not alter the form or magnitude of the orienting response (OR) to a novel olfactory stimulus (amyl acetate), nor does it affect the rate of response habituation. These results indicate that fundamental memory and attention systems are not disrupted by fetal alcohol exposure, an implication which challenges a well-established human literature of the cognitive and behavioral deficits associated with early exposure to alcohol. Insightfully, Hayne et al. suggested that the timing of ethanol exposure might be a crucial factor into modeling the cognitive deficits associated with fetal alcohol effects. Because the "brain growth spurt" occurs postnatally in the rat, perhaps if the animal were to receive ethanol exposure during this vulnerable period response habituations deficits would be observed.

This implication was addressed by prior work in our lab. Using a postnatal model of ethanol exposure, Hunt and Phillips (2003) demonstrated that ethanol insult during the third-trimester equivalent period produced significant deficits in response habituation to an odor while sparing the integrity of the orienting response. Indeed, this pattern of results was replicated by Hunt, Colona, Hruska, and Hillard (2001) who, in addition, demonstrated that the deficits in response habituation were specific to olfaction, as no habituation differences were detected when using an auditory stimulus. This finding was supported by Kelly and Richards (1998) exploration of

postnatal ethanol exposure on response habituation to auditory and visual stimuli in pre-weanling rats, the same age tested in our lab. Their results indicated that early alcohol exposure, in this case postnatal, does not disrupt attention, as measured by cardiac orienting and habituation.

The work of Hunt and colleagues (2003, 2001) indicated that postnatal binge-like ethanol exposure disrupts mechanisms of olfactory-based non-associative memory. Because olfaction is the primary sensory modality in rats, alterations in an animals' ability to learn about simple odors can impinge on other, higher order expressions of cognitive and behavioral development later in life. The purpose of Experiment 1 was to replicate the finding that postnatal alcohol exposure in binge-like doses during the third-trimester equivalent period induces dysfunctions in the rate of response habituation in the pre-weanling period. Based on prior work in this lab (Hunt & Phillips, 2003; Hunt et al., 2001), it was predicted that the integrity of the orienting response in both ethanol-exposed and sham-intubated animals would be maintained. Due to the strong evidence in the human literature indicating that alcohol-related cognitive and behavioral deficits last into and beyond childhood and adolescence (Coles, 2001; Streissguth et al.; 1983, Nanson & Hiscock, 1990; Carmichael-Olson, Feldman, Streissguth, Sampson, & Bookstein, 1998) it was hypothesized that alcohol-induced response habituation deficits in animals, previously observed on postnatal day 16 (Hunt et. al., 2001; Hunt & Phillips, 2003) would be present during the pre-weanling period and would persist into the post-weanling period (PD 23) and adolescence (PD 30).

Method

Subjects

The subjects were the 4 day-old offspring of 13 litters of Sprague-Dawley rats born and reared in the College of William and Mary Psychology Department's vivarium. On Postnatal day 2, the litters were culled to 10 animals (Day 0 equals day of birth). The mating pairs were housed in 48.0 cm x 25.5 cm x 20.5 cm polycarbonate maternity cages with wire lids and pine shavings. The dams were given ad lib access to water and Purina high-protein Rat Chow. The vivarium was maintained on a 12:12 hour light-dark schedule with light onset at 0700h. The College of William and Mary Research on Animal Subjects Committee approved the appropriation and treatment of the animals, as well as the present procedures.

Apparatus & Materials

Intragastric Intubations. The intubations involved a length of polyethylene tubing (PE-10, Clay-Adams, Sparks, MD) lubricated with corn oil (Rich food) attached to a 27 ½-guage needle on a 1 ml syringe. The concentration of the ethanol solution was achieved by combining 95% ethanol (Sigma Chemicals, St. Louis, MO) with baby formula (Similac with iron) to reach an 11.9% v/v solution.

Weight Measurement. An Ohaus top-loading balance (model GT 8000), accurate to the .01g, was used to assess the weight of the subjects.

Heart Rate Collection. Two transcutaneous electrodes were implanted into the skin of the subject to measure heart rate, one at the nape of the neck, the other 1cm above the base of the tail. The electrodes were connected to a preamplifier (Grass Instruments) by 32-guage Teflon-coated wires (Cooner Wires). After implantation, the subjects were placed inside a cylindrical Plexiglas container (14 cm

in diameter, and 25 cm in length) mounted horizontally inside a soundproof chamber (66 x 37 x 81.5 cm), which was lit by a 7.5 watt light bulb.

Changes in the electric potential between the two electrodes were recorded, before during and after stimulus presentation. From each electrode, the current flowed up the wires which were collected and stabilized at the lead, located at the top of the chamber. A computer recorded the intervals between R-spikes of each heartbeat to the nearest millisecond. Subject's cardiac signals were visually displayed using a Hitachi oscilloscope (model V-212). The intervals between R-spikes were recorded for 5 seconds before stimulus presentation, during the 10-second stimulus, and for 5 seconds after the offset of the stimulus. Ten trials were given to each subject.

Olfactometer System. A water bath, maintained at 40 degrees Celsius, was connected to a water-heated coil to transfer heat into the air stream. A Thomas air pump circulated the warmed air into the system, and the stream of air, controlled by two T-valves, was compressed through a flask containing 40 ml of water. When the 10-second olfactory stimulus began, the air was rerouted through a flask containing a solution of amyl acetate (0.5 ml amyl acetate and 40ml tap water). The air was continually pumped out of the chamber by the negative pressure created by an exhaust fan.

Procedure

Ethanol Exposure. Beginning on PD 4 and continuing through PD 9, pups were sexed, weighed, and marked for identification with a non-toxic permanent marker acutely before the first ethanol feeding. Subjects were randomly assigned to

one of 6 conditions: ethanol-exposed (tested on PD 16, 23, or 30), and sham control (tested on PD 16, 23, or 30). No more than two animals from each litter were assigned to each condition, and equal numbers of males and females were assigned to each group when possible.

The weight of each subject (g) was multiplied by 0.0279 ml/g to calculate the volume (ml) of alcohol/milk solution to be delivered during each intubation. This solution was made daily, refrigerated between uses, and was warmed just prior to administration.

For each intubation, the rats were removed from the dam and placed as a group in a 35.2 cm x 21.9 cm x 13.0 cm opaque polyethylene holding cage on a heating pad set to 34 degrees Celsius to maintain body temperature. The intubations involved inserting PE-10 tubing down the pup's esophagus and infusing the pre-measured volume of the ethanol/milk solution via a syringe attached to the tubing. The process took approximately one minute per animal. After each intubation, the pup was returned to the holding cage, and after all feedings were completed, the litter was returned to the home cage.

The ethanol (EtOH) exposed rats were administered a dose of 5.25 g/kg/day EtOH in milk solution (11.9% v/v) which was given in the first two daily intubations (1000, and 1200h). A third daily intubation (1400h) administered the milk vehicle (Similac) alone to supplement nutrition during peak intoxication. Sham-intubated controls were also given the three daily intubations, but no fluid was infused as, in prior research this produced abnormally heavy control animals (Goodlett & Johnson, 1997). After the last intubation on PD9, pups were earmarked to denote condition

and were returned to the home cage. On PD21, pups were weaned and group housed for the duration of the experiment.

Heart Rate Testing. On either PD16, 23, or 30 (+/- 1 day), pups were tested for cardiac orienting to 10 presentations of the 10-second olfactory stimulus (amyl acetate). Heart rate was recorded through two transcutaneous electrodes implanted acutely prior to test. Each animal was placed in the chamber for a 15-minute adaptation period (Saiers & Richardson, 1990) which was followed by 10 presentations of the stimulus separated by 100-200 second inter-trial intervals. After the end of the tenth trial, the electrodes were removed and the subject was returned to the home cage. Data were initially collected as inter-beat intervals (IBI) and later converted to beat-per-minute (BPM) measures of heart rate. The change in heart rate (relative to the pre-stimulus baseline) served as a measure of the orienting response. Changes in the magnitude of this response across trials were used to assess habituation.

Data Editing. Before analysis was conducted, IBI data for each animal were scanned for outliers and EMG artifacts. In this case, outliers were data indicating an IBI that did not fit within the physiologically realistic range, specifically 100-200 milliseconds. Next, IBIs were converted into mean heart rate change data using the IBIAN (Inter-beat Interval Analysis) program (Coulbourn Instruments). For every animal, this program generated a baseline measure of pre-stimulus heart rate (BPM) for each trial, as well as a second by second measure of heart rate change for each trial. This was conducted for each of the 10 trials (15 seconds per trial). Therefore,

the complete data set for each animal consisted of 10 baseline heart rate scores and 150 heart-rate change scores.

Results

Weight Analyses. Ethanol-induced changes in body weight during the ethanol administration procedure were analyzed by conducting a 2 (ethanol) x 6 (postnatal day) mixed design ANOVA (see Figure 1). Main effects of ethanol, F (1, 98) = 44.94, $p<.05$, as well as postnatal day, F (5,490) = 1350.27, $p<.05$, were revealed. In addition, a significant Ethanol x Postnatal Day interaction existed, F (5, 490) = 75.22, $p<.05$. As the animals aged, the differences in the body weight increased. The sham-control animals gained weight at a faster rate than the ethanol-exposed animals. Planned comparisons indicated that ethanol-induced body weight differences started on postnatal day 5 and extended throughout the duration of ethanol administrations (smallest F (1, 107) = 22.491, $p<.05$).

Ethanol-induced changes in body weight at the time of heart rate testing were analyzed by conducting 3 separate one-way ANOVAs examining the effect of ethanol exposure on body weight on PD 16, 23, and 30 (see Table 1). The analyses indicated an ethanol-induced body weight difference in pre-weanlings (PD 16), F (1, 26) = 4.64, $p<.05$. Just as was observed on PD9, the sham-control animals weighed more than the ethanol-exposed animals. A difference was observed in the body weight of post-weanling animals (PD 23), F (1, 22) = 3.25, $p<.05$. This would indicate that the impact of ethanol on body weight declined with age. Finally, no difference was observed between ethanol-exposed and sham-control animals on PD 30. This

indicates that by the time the animals reached adolescence, the effects of ethanol on body weight had dissipated.

Baseline Heart Rate. To assess differences in baseline heart rate, a 2 (ethanol) x 3 (age) x 10 (trial) mixed design ANOVA was conducted (See Figure 2-4).

There was a main effect of ethanol exposure on the baseline heart rate, F (1, 55) = 9.98, $p<.05$. Overall, ethanol-exposed animals had a higher baseline heart rate than the sham-controls. In addition, a main effect of age was revealed, F (2, 55) = 4.90, $p<.05$. With age, baseline heart rate decreased. Planned comparisons indicated that the baseline heart rate of pre-weanling animals was significantly different than adolescents F (1, 37) =10.85, $p<.05$. Also, there was a significant effect of trials on the baseline heart rates, F (9, 258) = 6.49, $p<.05$. This means that regardless of age or condition, baseline heart rate decreased with repeated stimulus presentations or time in the testing chamber.

The analysis of the Ethanol x Trial interaction yielded a significant difference, F (9, 258) = 2.58, $p<.05$, in baseline heart rate in response to repeated presentation of a stimulus, as a function of ethanol-exposure. Overall, baseline heart rate decreased more across trials in the sham-control group (as it should) than in the ethanol-exposed groups.

Cardiac Orienting. Differences in cardiac orienting to an odor were assessed for group by conducting a 2 (ethanol) x 3 (age) x 15 (seconds) mixed design ANOVA for average heart rate changes specifically on Trial 1, the first exposure to the novel odor (See Figures 5-7). There was a significant effect of ethanol on heart rate

changes during Trial 1, F (1, 55) = 2.20, $p<.05$. In addition, there was a significant effect of seconds, F (14, 176.25) = 6.04, $p<.05$. A significant Ethanol x Seconds interaction was observed, F (14, 176) = 3.32, $p<.05$. As the seconds of stimulus presentation increased, the heart rate of the ethanol-exposed animals decreased more than that of the sham-controls. No main effects or interactions involving age were found.

However, inspection of Figures 5, 6 and 7 suggested that for adolescents, in particular, the sham group was exhibiting a different pattern of cardiac change to the odor. We therefore conducted separate 2 (ethanol) x 15 (seconds) ANOVAs for each age. In pre-weanling animals (PD 16), there was no effect of ethanol on the expression or magnitude of the cardiac OR. A significant effect of seconds was observed on trial 1 for all animals, F (14, 266) = 6.863, $p<.05$. That is, as the duration of the stimulus presentation increased, heart rate typically decreased. After the stimulus was terminated, heart rate began to return to the baseline rate.

In addition, although the ethanol effect appears to be approaching significance, post-weanling animals (PD 23) demonstrated no ethanol-induced alterations in the expression or magnitude of the cardiac OR due to high variability in the sham-control group. Indeed, some of the sham-control animals actually showed increases on trial 1, a trend that will be discussed in more detail in regards to the adolescents. Again, a significant effect of seconds was observed on trial 1 for all animals, F (14, 266) = 6.863, $p<.05$. Just as the pre-weanlings demonstrated, as the duration of the stimulus presentation increased, heart rate typically decreased. After the stimulus was terminated, heart rate began to return to the baseline rate.

However, in adolescents (PD 30), a main effect of ethanol was observed on trial 1, F (1, 17) 6.81, $p<.05$, with ethanol-exposed animals exhibiting large negative changes in heart rate (indicative of orienting), and sham-control animals exhibiting no significant changes in heart rate. Indeed, in a pattern similar to the post-weanlings, many of the sham-control animals showed unexpected, but consistent increases in response to the odor on trial 1. Contrast analyses confirmed that during the first presentation of the stimulus, alcohol-exposed animal oriented and sham-control animals did not.

Response Habituation. In order to assess differences in response habituation in pre- and post-weaning animals, a 2 (ethanol) x 2 (age) x 10 (trial) x 15 (seconds) mixed design ANOVA was conducted. Because the animals tested during the pre- and post-weaning periods came from the same litters, it was appropriate to conduct an analysis for age-related differences in behavior as well as ethanol-induced change. Analysis of ethanol's impact on response habituation in adolescents was conducted separately.

The analysis for pre- and post-weanling animals demonstrated a main effect of ethanol, F (1.37) = 5.17, $p<.05$. Next, a main effect of trials was revealed, F (9, 333) = 2.94 $p<.05$. Finally, a main effects for seconds was also yielded, F (14, 518) = 29.81, $p<.05$. No effects or interactions involving age were found. In addition to these main effects, several interactions were observed. The Condition x Seconds interaction, F (14,518) = 2.55, $p<.05$, reveals that across the two ages, and importantly across all trials, ethanol-exposed animals displayed larger orienting responses to the increased duration of the odor being presented (see Figure 8). In

other words, regardless of how many times the odor had been presented, the longer the odor was present in the chamber, the more animals responded. The interaction indicated that throughout this stimulus presentation, ethanol-exposed animals showed a greater magnitude orienting response than did sham-controls.

Interestingly, there was an observed Age x Trial interaction, F (9,333) = 2.19, $p<.05$. This indicates that regardless of whether or not an animal was exposed to ethanol, older and younger animals responded differently across trials. Indeed, pre-weanling animals displayed orienting responses larger than post-weanlings during the first few trials, yet on all subsequent trials the older, post-weanling, animals displayed larger responses. Finally, the expected interaction of Trials x Seconds, F (126, 4662) = 1.88, $p<.05$, was observed. This interaction indicates that with repeated exposure to a stimulus, the second-by-second responses decreased.

Contrast analyses were conducted to determine the trial at which response habituation occurred. Although analyses were conducted using the mean change in heart rate, to simplify the presentation of the data, Figures 9 and 10 illustrate peak changes in heart rate. Habituation was defined as two consecutive trials of non-responding subsequent to (usually on trial 1) a significant orienting response. Analyses indicated significant orienting responses on all ten trials for pre-weanlings exposed to ethanol (smallest F (15,120) =3.27 $p<.05$) as well as the ethanol-exposed post-weanlings (smallest F (15, 135) = 1.82 $p<.05$). However, identical contrast analyses for the sham-control groups revealed that in pre-weanling animals, orienting responses occurred only during the first five trials (smallest F (15, 165) = 3.89 $p<.05$), followed by complete habituation of the cardiac response. In addition, the pattern of

response in the sham-control group tested during the post-weanling period was similar, however, in these animals, complete habituation occurred after the first four trials (smallest F (15,150) = 2.39 $p<.05$).

In adolescents, analyses revealed main effects for condition, F (1, 16) = 5.00, $p<.05$. Overall, ethanol-exposed animals responded to the odor with greater negative heart rate change than sham-control animals. In addition, a main effect of seconds, F (14, 224) = 8.90, $p<.05$, was yielded where, regardless of condition, as the duration of odor presentation increased, the magnitude of hear rate change increased.

In adolescent animals, there was an interaction of Ethanol x Seconds that was marginally significant, F (14, 42) =2.49, $p=.084$. As can be seen in Figure 11, across all trials, as the duration of stimulus presentation increased (from 1 to 15 seconds), ethanol-exposed animals showed consistently larger decreases in heart-rate than sham-controls. For age-comparison purposes, the Ethanol x Trial means are presented in Figure 12.

Discussion

The results of our examination of body weight and baseline heart rate are supported throughout the fetal alcohol literature, and although they speak to the reliability of this model, they will not be discussed. There were two purposes of Experiment 1. The first was to replicate this lab's previous finding that pre-weanling rats exposed to binge-like doses of ethanol during the brain growth spurt exhibit deficits in the rate of response habituation to an olfactory stimulus, without alteration of the initial cardiac orienting response. These effects were successfully replicated,

as all pre-weanling animals showed bradycardia (decreases in heart rate), the magnitude of which was a function of the duration of time the odor was presented.

The second goal was to determine the duration of these alcohol-induced alterations in response habituation. In pre-weanling rats, alcohol caused a decreased rate of response habituation. Whereas the sham-control animals showed no response to an odor after 5 trials, alcohol-exposed animals continued to show significant orienting responses upon every stimulus presentation. Post-weanling rats exposed to alcohol during the brain growth spurt showed a similar pattern as the pre-weanlings. Again, responses of the sham-control animals habituated (this time after 4 trials) yet the alcohol-exposed animals continued to exhibit responses throughout the testing procedure.

Even when controlling for baseline heart rate, one difference between the pre- and post-weanling expression of orienting and habituation in ethanol-exposed animals was the magnitude of the deficits. When tested during the post-weanling period, the magnitude of the alcohol-induced habituation deficit was dramatically larger than that of the pre-weanlings. Thus, with age, the nonassociative and short-term memory systems of these alcohol-exposed animals declined. Therefore, it appears that alcohol-induced changes in habituation of the cardiac OR are a persistent problem throughout development.

Just as Streissguth et al. (1983) demonstrated with their study of infant habituation, orienting responses were intact for the alcohol exposed animals. The difficulty was in habituating this response. All ethanol-exposed animals showed deficits in response habituation. Of particular interest was the increased magnitude of

the responses demonstrated on the later trials by the 23-day-olds. This indicated that, with age, the habituation deficits increase, and that although all the alcohol has long been eliminated from the system, the deleterious effects of postnatal alcohol exposure continued to affect the development of the CNS and expression of attention and short-term memory.

In the human literature, cognitive effects of fetal alcohol exposure are evident beyond childhood and into adolescence. Brown, Coles, Smith, Platzman, Silverstein, Erickson, and Falek (1991) noted impairments in attention and STM in 12-14 year old children who were prenatally exposed to alcohol. Carmichael-Olson, Feldman, Streissguth, Sampson, and Bookstein (1998) conducted a thorough investigation of the neuropsychological deficits in adolescents with FAS. These patients commonly show behavioral and learning problems, decreased social skills, and impaired adaptive behaviors. Carmichael-Olson et al. (1998) found that significant attention deficits were present (specifically shifting and encoding attention).

Kerns, Audrey, Mateer, and Streissguth (1997) discussed the cognitive deficits in non-retarded adults with FAS. Regardless of IQ, these patients demonstrated clear deficits on measures of attention (specifically sustained), auditory learning and executive function. In a study examining the individual differences in auditory and visual attention among fetal alcohol affected adults, Connor and Streissguth (1999) found that the attentional disturbances observed for children with FAS/FAE do not dissipate, as is often the case with facial malformations. This study revealed significant deficits on the focusing, shifting, and sustaining components of auditory

attention, and on focusing and sustaining visual attention. Encoding, however, was not examined in this study.

Due to the empirical evidence of alcohol-induced deficits in attention and short-term memory, it was essential to continue to extend testing of the current animal model to address the deleterious effects of alcohol in the later stages of development. When tested during adolescence (just one week after the post-weanling period), the alcohol-exposed animals followed the same trend of response habituation deficits as the younger (pre- and post-weanling animals). However, the sham-control animals never once displayed an orienting response to the odor. This unexpected result makes any implications based on these data tenuous. Yet interestingly, when examining the raw, unaveraged heart-rate data for the sham-control animals, an unusual pattern was detected.

In all prior research in this lab, including the pre-and post-weanlings and alcohol-exposed adolescents of the current study, all animals displayed consistent and robust negative heart rate changes elicited by the presentation of the odor. They exhibited an orienting response. This was especially the case on trial 1, which was the very first exposure to amyl acetate. As a group, the sham-control adolescents did not. Interestingly, it was observed that some of the adolescent sham-control animals displayed these robust negative heart rate changes, yet the majority of these subjects exhibited dramatic increases in heart rate (see Figure 13); responses which are typically indicative of increases in defense-related behaviors (Graham, 1992). According to Graham, by definition, an orienting response occurs when heart rate decreases and movements are reduced. In addition, orienting responses rapidly

habituate, and learning is promoted. However, Graham defines the defensive response as increased heart rate, coupled with increased motor activity. This response habituates slowly, and learning is impaired.

When averaged, it appeared that there was no response in our sham-control group. However, the data suggest that two competing responses might be simultaneously elicited, but only one response, either orienting or defensive, can be expressed. Although odor elicited activity in the testing chamber was not directly measured, Spear (2000) indicated that adolescent animals have been shown to react to experimental context with greater levels of stress than both older and younger animals. Spear notes that although it may seem paradoxical for adolescent animals to behave in ways that demonstrate both risk taking and increased stress to novel environments, in evolutionary terms, this shifting between orienting and defensive behaviors is adaptive, as it acts to develop the vigilance necessary for survival beyond the home nest.

It is therefore possible that the sham-controls are responding just as they should. The possibility that the lack of an orienting response and the presence of defensive behavior is the normal response for adolescents combined with the pattern of similarity between the alcohol-exposed animals in the pre- and post-weanling periods and the alcohol-exposed adolescents indicates that the alcohol-induced differences might be due to a developmental delay (Riley, 1990).

In the animal literature, there are several examples of effects of alcohol that are observed in younger animals but appear to diminish as the animal matures (Riley, 1990). The transient nature of some of the neurobehavioral effects has led to the idea

that alcohol exposure results in developmental delays. In other words, when a young animal is tested, the effects observed might be due to immaturity in the animal's response mechanisms. As the animal matures and catches up in development, the differences from controls can diminish. This would indicate that the animal has learned or developed compensatory mechanisms or strategies for dealing with these dysfunctions (Riley, 1990).

According to Means et al. (1984) rat pups exposed to alcohol prenatally are typically hyperactive compared to controls. However, as they mature and reach adulthood, this hyperactivity diminishes. In addition, alcohol-related deficits on simple learning tasks such as passive avoidance learning, are observed around the time of weaning, but were not found in older animals (Hannigan et al., 2000). Finally, data have been reported demonstrating that alcohol exposed animals, previously shown to display poor performance on the 8-arm radial arm maze, performed on PD90 at the same level observed in normal young rats (Riley, 1990).

Future examination of this potential developmental delay demands two subsequent experiments. To establish that the normal response of an adolescent rat is to not orient to odor presentation, one would need to test the OR and response habituation of animals with no experimental experience. This study is currently underway. In addition, to determine that the alcohol-induced effects in adolescents are demonstrative that they are responding as younger animals, one would need to test the alcohol-exposed animals at a later age and compare their pattern of response, possibly during early adulthood, to the control responses during adolescence.

Although it cannot yet be firmly established whether alcohol-induced deficits in response habituation are a persistent problem or a developmental delay, certain assertions can be made with confidence. Alcohol does not affect the ability of an animal to orient to a stimulus, as noted by intact orienting responses. However, alcohol does appear to interfere with the animals' ability to establish a memory for the odor. Olfactory-based attention mechanisms, as measured by the lack of response habituation, are damaged in these ethanol-exposed animals.

Disrupted ability to attend to and learn about a novel odor in the laboratory setting can have strong implications regarding other olfactory based learning and memory mechanisms. The primary sensory modality for rats is olfaction. In the developing rat, intact olfactory learning abilities are critical for survival; they allow the animal to locate the home nest, attach to nipples to suckle, and to identify other animals as familiar (i.e. the dam and littermates) or unfamiliar (i.e. an intruder) (Friedman & Rosenblatt, 1978).

For rats, conspecific recognition is olfactory-based (Thor & Holloway, 1982). Any disruption in the ability to appropriately use odor cues (be it a lack of response to another animal, or the persistent attention to another animal) can impact the quality of social interactions. Similar disruptions in social behaviors have been consistently reported in adolescents diagnosed with FAS (Streissguth et al, 1991; LaDue et al, 1992). Carmichael-Olson et al. (1998) showed that FAS patients were rated as having significant problems in adaptive behavior, specifically in social competence, and over half the group reported behavior problems, and troubled performance in school. In addition, using the Vineland Adaptive Behavior Scales (VABS), Whaley,

O'Connor, and Gunderson (2001) found that children who were exposed to alcohol showed deficits in all domains of adaptive functioning, including communication, socialization, and daily living skills. In addition, the authors posited that deficits in socialization especially may become more significant with age, perhaps upon adolescence.

Experiment 2. Fetal Alcohol Effects and Adolescent Social Interactions

Adolescence has been shown to be a period of transitions in other species as well as humans. Aside from the biological changes associated with passing through puberty, it is a time marked by both qualitative and quantitative changes in social behavior. The development of social behavior during adolescence takes the form of learning and practicing mock forms of both sexual and aggressive behavior. Pellis & Pellis (1987) showed that around the time of weaning, rats display a repertoire of defensive behaviors similar to that of adults. However, later in development, adolescent rats exhibit frequent and intense bouts of rough-and-tumble play fighting. This play behavior is a hallmark of adolescence, and diminishes with the completed transition to adulthood, as marked by sexual maturity (Panksepp, 1979). After adolescence, the adult-like repertoire of aggression-linked defensive behaviors is evident once again.

In his discussion of the ontogeny of play, Panksepp (1980) indicated that although play behavior begins to emerge around 18 days of age, by day 25 the young adolescent has developed a complete array of play behavior including running, jumping, climbing, and initiating intense sham fights, which, as Small (1899) indicated, "always in the absence of fear". This play behavior is distinct to adolescents and peaks between day 30 and 40 (Panksepp, 1980). Evidence supporting the emergence of play as an innate pattern of behavior is particularly telling, as rats reared in an environment lacking conspecific social interaction (a

situation typically predicting altered subsequent social competence) still exhibited robust levels of play behavior upon peer encounter during adolescence (Ikemoto & Panksepp, 1992).

Panksepp (1979) operationally defined play as the frequency and duration of pinning behavior. Pinning occurs when one animal pushes another onto its dorsal surface and holds it down with both paws. Although one result of pinning is that is aids in the development of stable dominance hierarchies (one animal will usually emerge as dominant and initiate 70% of pinning), the submissive animal in the pair always remains engaged in social interaction after being pinned (Panksepp & Beatty, 1980).

During adolescence, social interactions, in particular play, are crucially important. Panksepp and Beatty (1980) demonstrated that the satiety and deprivation functions of pinning were as systematic as those obtained with feeding and drinking which suggests that play is a regulated process. Although pinning has been determined as the best single measure of play behavior, Panksepp & Beatty (1980) identified several behavioral correlates of pinning that should be included in a complete analysis of play behavior. These include a positive correlation with following behavior, and a negative relationship with social investigative behaviors, such as grooming and social sniffing. It would appear that identification of who to interact with and the initiation of these solicited interactions operate using independent mechanisms.

Social recognition, one of the most biologically relevant and fundamental types of memory processes, can be defined as a decrease in social behaviors after

repeated exposure to another animal (Thor & Holloway, 1982). In order for this process to occur, the animal must form a memory of the initial encounter, and be able to recall and compare that memory during a second interaction. Social recognition is marked by a once novel animal becoming familiar; in essence, it is social habituation and is dependent on olfactory cues.

This behavior can be empirically tested using a naturalistic and elegant procedure developed by Thor and Holloway (1982). In this paradigm, an adolescent rat interacts with an adult male for two periods of five minutes each, separated by a varying interval of time. During the second exposure, when the same juvenile is reintroduced, the amount of the adult's investigation time represents the presence and strength of the memory of the first encounter. If the adult investigates during the second interaction significantly less than during the first exposure period, then it is assumed that a memory was formed and social recognition has occurred. However, if the adult investigates at the same frequency and duration as during the initial exposure, this is evidence of a lack of memory for the previous social encounter. Thor and Holloway demonstrated a direct relationship between investigation time during the second interaction and length of the inter-exposure-interval (IEI), noting that the social memory of the adult was lost 80 minutes after the first exposure.

Kelly and Tran (1997) used an adaptation of Thor and Holloway's procedure to examine the effect of early postnatal ethanol exposure on the social recognition behavior of adult male rats. In this study, Kelly and Tran administered binge doses of ethanol during the period (PD 2-10) of development equivalent to the late second trimester and all of the third trimester of human gestation. As previously described,

administration of high doses of ethanol during this early postnatal period is associated with severe cognitive and behavioral deficits. However, Kelly and Tran found only a marginal effect of ethanol on social recognition in adults and only after an inter-exposure-interval of 90 minutes.

In his discussion of developmental delays, Riley (1990) suggested that testing adults might not be a good indicator of behavioral and cognitive problems present earlier in life. That is, if behavioral or cognitive impairments due to early alcohol-exposure are present during infancy or childhood, the proper way to assess whether they represent permanent dysfunctions or developmental delays would be to test throughout the lifespan, and not just during adulthood. Neither in the human condition nor in the animal model of fetal alcohol effects, has it has been firmly established whether the cognitive and behavioral deficits associated demonstrate permanent dysfunctions or developmental delays. Indeed, evidence from Experiment 1 attests to both possibilities. However, as longitudinally-studied populations of humans with FAE age, more and more is being learned about the long-term effects of early exposure to alcohol.

Thor and Holloway examined the application of their paradigm to adolescent-adolescent interactions, but noted that the battery of intervals needed to be scaled down to the order of 1-8 minutes (as opposed to 10-80) because no social memory was observed in adolescents even with the smallest (10-minute) interval. They found that adolescent-adolescent social recognition was demonstrated only after very brief intervals (1 to 2 minutes) and that memory was gone by the 4 and 8 minute intervals. In terms of this methodology, adolescent social recognition memory was lost between

2 and 4 minutes after the initial interaction.

Because of the strong (albeit sparse) evidence for the deleterious effects of early exposure to alcohol on human adolescent social interactions, it was not only appropriate, but necessary to determine if adolescent social interactions (especially play behavior) were disrupted in the current animal model of fetal alcohol effects. Because of the social competency deficits seen in humans with early exposure to alcohol (Streissguth et al, 1991; LaDue et al, 1992, Carmichael-Olson et al., 1998) the pattern of social recognition, defined as differences in proportional scores of play behavior as a function of interval length, was hypothesized to be disrupted in alcohol-exposed adolescent animals.

Methods

Subjects

The subjects for this study were the offspring of 9 litters of Sprague-Dawley rats born and reared in the Psychology vivarium at the College of William and Mary. Five of the litters were used in the alcohol exposure procedure; the other 4 supplied the naïve social stimuli. All animals were born and reared under the previously described conditions at the College of William & Mary. On PD21, pups were weaned and group housed. On PD 29, animals were individually housed and with ad lib access to food and water for the duration of the experiment.

Apparatus

Social Recognition. The chamber for the social interactions was a 54 cm x 41cm x 31cm plastic Tupperware bin with pine shavings covering the bottom. Background illumination was supplied from two lamps with 25 watt red light bulbs.

Interactions were recorded with a Panasonic video camera; model TRV608, which was mounted approximately 120 cm above the test chamber.

Procedure

Alcohol Exposure. The postnatal alcohol exposure by means of intragastric intubations was used. For a description, see Experiment 1.

Social Recognition Testing. On PD 30 EtOH and Sham animals were assigned as subjects (SJ) and experimentally naïve animals were assigned to the social stimulus (ST) condition. All animals were marked with a non-toxic permanent marker; the subjects had dots on the back of the head, and the stimuli had a stripe down the back. All animals were transferred to the testing room in individual holding cages. Interaction pairs consisted of same sex animals; one subject (either EtOH or Sham) and one naïve stimulus. Because the hypothesized memory span for these animals was on the order of minutes, the same pairs were used throughout testing.

The recognition procedure consisted of two interaction periods separated an interval of varying length. Animals were placed in the chamber and allowed to interact for 5 minutes. They were then removed from the interaction context and placed in their respective holding cages for a timed interval of 1, 2, 4 or 8 minutes. Next, they were reintroduced and allowed to interact again, this time, in an identical bin with fresh shavings. After each set of interactions was over, the pair was returned to their individual cages for 24 hours until the next interaction. The schedule of 4 intervals x 4 days was counterbalanced, and all pairs were tested on each interval, but not more than once in 24 hours.

Behavioral Coding. All measures were coded for the subject only. Behavior of the ST animals was never explicitly observed. For both interactions, the frequency of several behaviors was recorded. Sniffing (SNF) was defined as the subject nasally exploring the ano-genital region of the stimulus animal; grooming (GRM) occurred when the subject licked and pawed at the stimulus animal; boxing (BOX) was noted when the subject stood upright on its hind legs and pawed with its forearms at the stimulus animal; following (FOL) occurred when the subject walked behind, but in close proximity to the stimulus animal for at least one length of the chamber; pinning (PIN) was defined as the subject pushing the stimulus animal onto its dorsal surface and holding it down with both paws.

Results

The mean proportional scores for each behavior and interval are displayed in Figure 14. Although all measures of social behavior were coded, the statistical analyses focused solely on proportional differences in play behavior across intervals as a measure of social recognition.

Play Behavior. For the purpose of this experiment, play behavior was defined as the combined total of following and pinning. Pilot work (Colona, 2002) indicated that these two behaviors were highly correlated and were the best indicators of social play behavior in subjects of this age. For statistical analysis, a proportional score (frequency of play behavior during the post-interval interaction/ frequency of play behavior during the pre-interval interaction) was calculated. By this method, a proportional score of 1.0 indicated no change in play during the second interaction (i.e. forgetting) and proportional scores less than 1.0 were used to infer recognition.

A 2 (ethanol) x 4 (interval) mixed design factorial ANOVA was conducted on the proportional scores for play behavior (see Figure 14). A main effect for ethanol was observed, F (1, 18) = 7.08, $p<.05$. This means that overall, ethanol-exposed animals (M= .65, SEM= .05) exhibited more play than sham-control animals (M= .49, SEM=.04). In addition, a main effect for interval was observed, F (3, 54) = 6.05, $p<.05$. For both conditions, as the interval of time between interactions increased, the amount of play also increased (1-minute interval: M= .41, SEM= .06; 2-minute interval: M= .47, SEM= .07; 4-minute interval: M= .65, SEM= .06; 8-minute interval: M= .75, SEM= .07).

Although there was not a significant Ethanol x Interval interaction, inspection of the figure suggested that the EtOH subjects exhibited forgetting at the longer IEI's and that the Shams did not. Planned comparisons indicated that there was a significant difference between ethanol and sham subjects at the 8-minute interval, t (18) = 2.12, $p<.05$. Within group comparisons using the 1-minute IEI as a baseline indicate that play was significantly increased after both the 4-minute interval (t (9) =2.86, p<.05) and the 8-minute interval (t (8) = 3.59, 0<.05) within the ethanol-exposed animals. However, within the sham-control animals, neither play after the 4-minute interval nor the 8-minute interval significantly differed from that of the 1-minute interval.

Discussion

Experiment 2 revealed that postnatal binge-like exposure to ethanol during the brain growth spurt increases play behavior in adolescent rats. In addition, social recognition was altered in ethanol-exposed animals. The finding that young animals

play more after periods of social isolation is supported in the literature, (Panskepp, 1980), as are alcohol-related increases in global play (Meyer & Riley, 1986).

In terms of social recognition behavior, Kelly and Tran (1997) reported finding only a marginal effect of postnatal alcohol exposure on social recognition in adult rats. In the current exploration of adolescent social recognition, a similar trend was detected, in that only during the longest interval of time between interactions was an ethanol-related difference evident. Whereas sham-control animals demonstrated some retention of the social memory during the longer intervals, the ethanol-exposed animals clearly did not.

One major difference between the current experiment and Kelly and Tran's work was the operational definition of the behaviors measuring social recognition. Kelly and Tran chose to define social recognition as the proportional differences in investigative behaviors. With this definition, the results from Experiment 2 showed no effect of alcohol or interval on differences in social behavior. Yet clearly, as was observed by the effect of alcohol on overall play, there is more governing the quality of social interactions than investigative behaviors.

Our finding of altered social behavior is well supported in the human literature on fetal alcohol exposure. Thomas, Kelly, Mattson, & Riley (1998), used the Vineland Adaptive Behavior Scale (VABS) to identify that periadolescents with FAS had deficits in social skills of approximately three standard deviations below the mean for their age. In addition, adolescents with FAS or FAE show a lack of reciprocal friendships, lack of trust, and difficulty in cooperating with peers (La Due et al., 1992).

The lack of reciprocal friendships is another social dysfunction that could be tested with an animal model of fetal alcohol exposure. Although it was not formally examined, casual observations indicated that some of the subjects seemed to persistently try to interact with the stimulus animal in Experiment 2, even when the stimulus animal was immobile in the corner of the testing chamber. This could be related to the observation in human adolescents with fetal alcohol exposure have trouble in the maintenance, but not the initiation of social relationships (La Due et al, 1992). Perhaps the reason these individuals have such unstable social functioning is not due to their termination of social relationships, but the other partner of the dyad ending social interaction due to the social relentlessness of the FAE individual. This hypothesis can be tested by coding the behaviors of the stimulus animal, in addition to pair behaviors, such as together time, to examine the global social functioning of animals exposed to alcohol. Thus, the control afforded by development of an animal model would allow study of naturalistic social interaction dyads, something that has rarely been examined in the human condition.

Adolescents diagnosed with FAS/ARND demonstrate not only altered patterns of social behaviors, but also engage in high risk behaviors. Using the Leading Health Indicators scale (LHI), Streissguth, Barr, Kogan, and Bookstein (1997) demonstrated that starting at the age of 12, individuals diagnosed with FAS were more likely to have disrupted school experiences, more trouble with the law, and strikingly, many of the patients in the sample (30%) were reported to have experienced problems with drug or alcohol abuse.

In a recent review of the adolescent brain and behavior Spear (2000) indicated that adolescence is a transitional period of life in human development characterized by increased emphasis on social relationships, increased risk taking, cognitive development and experimentation with drugs and alcohol. In addition, is has been well documented that early experiences with alcohol can lead to alcohol use and dependence later in life (for a review, see Spear, 2000). Indeed, the 1992 National Longitudinal Alcohol Epidemiological Survey of current and former drinkers indicated that the rate of lifetime alcohol abuse was 40% when drinking began during or before adolescence (Grant & Dawson, 1997). However, only 10% of lifelong drinkers started drinking after age 20. Thus, it is evident that early experiences contribute to later alcohol preferences.

Experiment 3. The effects of early experience with ethanol on preferential ethanol intake during adolescence

To date, little research in the human or animal literature has explicitly investigated the role of fetal alcohol exposure in the etiology of later alcoholism. However, Baer, Barr, Bookstein, Sampson, and Streissguth (1998) have provided data from a 14-year longitudinal study (known as the Seattle Longitudinal Study) pertaining to the relative contribution of fetal alcohol exposure in the prediction of adolescent alcohol problems. In this study, 14-year-olds with known prenatal exposure to alcohol provided information about the frequency and quantity of alcohol consumption within the past month and the consequences of their drinking over the past three years. The data revealed that 57% of adolescents with prenatal alcohol exposure reported drinking, 25 % within the past month. Of adolescents who drank within the past month, most reported that, when drinking, they would ingest 1 to 2 drinks. However 21% of these individuals reported drinking 3 or more drinks per episode, and 31% of all adolescents reported having at some time felt intoxicated from alcohol. Baer et al. (1998) concluded that prenatal exposure to alcohol was more predictive of adolescent alcohol use and its negative consequences than was family history of alcohol problems. These data suggested that adolescents with fetal alcohol exposure have greater involvement with alcohol and more associated negative consequences.

To this author's knowledge, no examination of these adolescent drinking patterns related to fetal alcohol exposure has been conducted with animals. Experiment 3 examined the role of postnatal alcohol exposure on alcohol intake during adolescence.

Methods

Subjects

Subjects for this study were the 4-day old animals of 11 litters of Sprague-Dawley rats housed and reared under the conditions described above. Subjects were assigned to one of three groups. The ethanol-exposed and sham-control groups were comprised of the subjects previously tested in Experiment 1, which investigated the effects of postnatal binge alcohol exposure on response habituation during adolescence. A third, experimentally naïve, group was formed from the offspring of 5 litters of Sprague-Dawley rats. No more than two animals from each litter were assigned to each group, and when possible, equal numbers of males and females were assigned to each group. On PD21, pups were weaned and group housed. On PD 32, pups were individually housed for the duration of the experiment.

Apparatus

The intubation materials were the same as those previously described for Experiment 1. Intake testing occurred in individual stainless steel hanging cages, located in a room adjacent to the vivarium, which was maintained on the same light/dark cycle and temperature as previously described. Intake was measured from 50-ml graduated boroscilate drinking tubes with curved sipper spouts. Both tubes were attached to the outside of the cages.

Procedure

Alcohol preference testing was a variation of the methodology described by Hunt et al. (2001). On PD 32, animals were individually housed in hanging cages described above. The subjects had ad lib access to food and water. On PD 35, subjects were presented with two graduated drinking tubes, one containing a 6% v/v EtOH solution dissolved in tap water, the other containing an equal volume of 1.5% v/v Sanka decaffeinated coffee solution dissolved in tap water. Placement of solutions (left vs. right) was counterbalanced for condition and gender to avoid a side-preference bias. Subjects were allowed to ingest solutions for 24 hours, at which time (on PD 36), intake measurements (in ml) were recorded from each graduated tube.

Results

To determine the effect of early experience with alcohol on intake during adolescence, analyses were completed for two measures of interest: 1.5 % coffee intake, and 6% ethanol intake. Figure 15 displays the alcohol exposure condition effects on intake of each fluid.

Analyses revealed a marginal effect of postnatal ethanol exposure on total fluid intake, F (2, 28) = 2.77, p=.08. Ethanol-exposed animals drank the most, followed closely by the sham controls, and finally by the naïve-controls. In addition, there was no significant effect of ethanol-exposure on the level of coffee intake (mean coffee intake was 7.97 ml, SEM= 2.00), indicating that coffee was equally ingested regardless of prior ethanol-exposure, or experimental treatment.

There was a significant effect of experience with alcohol on the level of ethanol intake, F (2, 28) = 7.69, p<.05. Post Hoc analyses indicated that although ethanol intake by the ethanol-exposed group was not significantly different from that

of the sham-control group, both groups were ingested significantly more than the naive control group, $t(17) = 3.947$, $p<.05$; $t(20) = 2.345$, $p<.05$, respectively.

Discussion

Experiment 3 indicated that ethanol-exposure does not impact the total fluid intake or preferential intake of coffee. In addition, it would appear that the direct exposure of an animal to the intoxicating effects of alcohol through intragastric intubations has the same effect as sham-control intubations independent of ethanol involvement. In addition, one might interpret that prior testing or experimental treatment caused this increase in ethanol ingestion. However, two alternative explanations for these results will be discussed, first regarding the ethanol-exposed animals, then addressing the intake pattern of the sham-controls.

The results of Experiment 3 revealed that animals exhibited a preference for an ethanol solution after early exposure to high doses of ethanol. This contradicts the finding of Molina, Chotro, and Spear (1989) who investigated early learning about the orosensory cues of alcohol due to detection of alcohol on the pre-weanling rat's own breath and saliva. In this case, alcohol was administered intragastrically and acutely to 11-day-old pups at a dose of 3.0 g/kg. One day later, when tested for preference for alcohol odor, subjects expressed a strong aversion to the orosensory cues of alcohol by spending significantly more time near a lemon scented cotton ball rather than the alcohol-scented cotton. This result suggests that although intragastric administration of alcohol bypasses the mouth, respiratory elimination of ethanol can provide powerful information for the animal to learn about ethanol's chemosensory qualities and associate these with aversive post-ingestion consequences of ethanol

intoxication. Strikingly, this aversive pairing did not occur in Experiment 3, as the ethanol-exposed animals demonstrated an appetitive response to presentation of ethanol solution by ingesting more alcohol than naïve controls. These findings are supported by the evidence that human adolescents (with fetal alcohol exposure), despite awareness of the aversive consequences of their alcohol-related behavior (from intoxication to psychosocial consequences), continue to consume alcohol (Baer et al., 1998).

It was striking that the ethanol-exposed animals demonstrated an increased preference for ethanol despite the fact that the dosage administered to these animals (5.25g/kg/day) was almost double the dosage Molina et al. found was aversive (3g/kg). In addition, the ethanol-exposed animals in Experiment 3 were delivered this binge-like dose for six consecutive days, whereas Molina et al.'s animals received only one exposure to ethanol. Thus, according to Molina et al., ethanol-exposed animals in this experiment should exhibit an aversion to the ethanol solution. Instead, they showed a preference for it. However, recent evidence has demonstrated that the salience of the home cage environment, especially the impact of social interactions within this context, acts as one of the most powerful positive reinforcements for otherwise aversive situations, potentially buffering the aversive properties of ethanol intoxication (Hallmark & Hunt, 2001).

Increased ethanol ingestion in sham-control animals was a surprising result. However, the following discussion of the social transmission of ethanol preferences provides an explanation for this observed trend in behavior. Because 10-12% of a given dose of ethanol in rats is not metabolized, but is eliminated through routes such

as respiration, salivation, urination, and perspiration (Goldstein, 1983), the chemosensory detection and learning about ethanol is possible both for the intoxicated animal and other animals in close contact. It is then possible that reinforcement due to littermate interactions in the home cage following acute intragastric administration of ethanol could serve as powerful rewards which could be associated with the respired ethanol. Therefore, the sham-control group in Experiment 3 might have established a positive association between the home cage context (specifically interactions with an intoxicated sibling) and the chemosensory qualities of ethanol, an association that was so strong, it overrode the natural aversion to ingest an ethanol solution.

Recently, Hunt, Holloway and Scordalakes (2001) developed a procedure to enhance voluntary alcohol intake in periadolescents (ranging from 25-36 days old), a variation of Galef et al.'s (1985) demonstrator-observer paradigm for social communication of diet preferences (for a review, see Hunt & Hallmark, 2001). According to Galef, after one animal (the observer) smells a novel food on the breath of another animal (the demonstrator) subsequent food choice will be affected. In their study, Hunt et al. established that even after brief (30 minute) interactions with an intoxicated sibling, preferential intake of alcohol (vs. coffee) in naïve observers increased significantly when tested immediately (compared to water and coffee exposed controls). In this case, an association was presumably made between respired alcohol and social reinforcement by interaction with a littermate, a powerful reward for animals individually housed.

Work with pre-weanling animals indicated that animals as young as 8 days of age (to date, the youngest age tested), after interacting with intoxicated siblings, showed greater ethanol ingestion than controls (Hunt et al., 1993; Hunt, Lant, & Carroll, 2000). This suggests that the sham-intubated animals in Experiment 3 were capable of learning about alcohol on the breath of a sibling during the time of the ethanol administrations (PD 4-9). In another experiment, Clary and Hunt (1999) found that multiple demonstrators of alcohol in the context of the home cage caused observers to ingest more alcohol than controls. In addition, multiple exposures to multiple intoxicated siblings within the context of the home cage (Hallmark & Hunt, 2001) induced a robust increase in ethanol intake (four times as much as controls), an effect that has been shown to last up to one week (Hunt et al., 2000).

In the context of the home cage environment following daily ethanol intubations on PD 4-9 (the alcohol exposure procedure), sham animals encountered multiple demonstrators which were intubated with alcohol twice per day, over several consecutive days. In other words, the sham-control animals were bombarded with social-based information about ethanol during a period of time in which they could, and did, learn about food preferences. Couple this with the positively-reinforcing environment of the home cage. The result is socially-mediated transmission of alcohol preferences in sham-control animals.

One surprising result of Experiment 3 was that olfactory memory due to early exposure to alcohol, either directly through ethanol intubations or socially transmitted on the breath of a sibling, was retained for at least 26 days (the last exposure to ethanol was on PD 9, and test occurred on PD 35). This long of a retention interval is

unprecedented in the literature on appetitive behaviors; prior to these results, the longest documented retention of socially-mediated alcohol preferences was 6 days (Hunt et al., 2000) not several weeks, as observed here. Further investigation of long term-retention after multiple exposures to alcohol is necessary and important to fully understanding the impact of early exposure to alcohol on adolescent drinking patterns.

One potential limitation of this experiment was that the subjects had been previously tested. Although this practice is common in research with animal models, especially those conducting preliminary investigation of a phenomenon, it is essential that this experiment is replicated with untested animals. In addition, because both the ethanol-exposed and sham-control animals ingested more ethanol, a potential confound exists. The cause of this pattern of effects could be that the intubation procedure, regardless fluid delivery, increases ethanol ingestion during adolescence. However, no prior work in this lab supports this pattern where both ethanol-exposed and sham-control animals demonstrate behavioral deficits. To explicitly test this, naïve littermates of the ethanol-exposed and sham-control animals should be tested to examine the sole effects of social transmission of alcohol preferences independent of the intubation procedures.

In summary, the results of Experiment 3 may indicate that early experience with alcohol, whether through direct exposure (ethanol intubations), or indirect exposure (ethanol-exposed and sham-intubated controls interacting with intoxicated siblings), produces a relatively long-term chemosensory-based memory which affects

preferential ethanol intake during adolescence. The implications of this work for studying human patterns of adolescent drinking are numerous.

With the knowledge that adolescents with fetal alcohol exposure are at a higher risk for developing substance abuse disorders during adolescence, medical professionals and parents alike may be able prevent, or at least be more conscious of the threat that drugs and alcohol pose to these alcohol-exposed adolescents. In addition, the possibility that alcohol preferences can be transferred on the breath of a significant other, has staggering implications for the environmental components of the etiology of alcoholism.

General Discussion

Profound consequences of early exposure to alcohol are present during adolescence and pervade multiple aspects of behavioral development. In humans, adolescents with known fetal alcohol exposure exhibit severe alterations in learning and memory, attention, social competency, and they are at a greater risk for developing substance abuse disorders. Each of these alterations in behavioral development has been demonstrated using our model of third-trimester alcohol exposure in the adolescent rat. Early postnatal binge-like ethanol exposure induced developmental changes in response habituation, altered social behavior, and an increased preference for ethanol in a free-choice situation. Together, these findings provide a well-rounded model of the human expression of fetal alcohol-related behavioral dysfunctions evident in adolescence.

First, we examined the effect of ethanol on response habituation, a fundamental form of nonassociative memory which is critical for more complicated forms of attention and associative learning. We found that adolescent rats exposed to alcohol during the neonatal period show a slow rate of response habituation. In combination with the evidence of similar alterations in pre- and post-weanling animals, we can assert that these nonassociative memory deficits likely represent a potentially permanent behavioral dysfunction.

After we established that the ability of an adolescent rat to learn about an odor (in a very fundamental fashion) was compromised, we decided to test this behavioral

deficit in another domain, social functioning. Because the tests of response habituation and social interaction in rats are olfactory-based, we expected to see similar dysfunctions in the ethanol-exposed animal's ability to display that a social memory had been formed. This is exactly what was observed. Not only did alcohol increase the frequency of play behavior in adolescent rats exposed to alcohol, but it caused a difference in the way these animals interacted with a conspecific, specifically after longer periods of time between social interactions. In other words, a familiar conspecific rat became unfamiliar again more quickly for the ethanol-exposed animals than the controls.

In a recent review of the effects of prenatal alcohol exposure on social behavior in humans and other species, Kelly, Day, and Streissguth (2000) stated, "The social problems (in adolescents with fetal alcohol exposure) were almost as prevalent as the attention problems.", (p.145). We do not think this is just a coincidence. According to the human fetal alcohol literature pertaining to adolescents, social behavior seems to be one facet of a large spectrum of adaptive behaviors disrupted by fetal alcohol exposure (as tested with the VABS and LHI).

Carmichael-Olson et al. (1998) noted that fetal alcohol exposure should be considered as a critical component in a developmental trajectory toward alcohol problems involving school failure, association with deviant peers, impulsive behavior, and criminality. Indeed, delinquency research implicating the contributory effects of impaired attention and hyperactivity on impulsiveness indicated that impulsive behavior was the best predictor of early delinquency (Tremblay, Pihl, Vitaro, & Dobkin, 1994). It therefore seems more than a coincidence that the cognitive

dysfunctions apparent in adolescents with FAE are so strongly associated with maladaptive social behavior. As was discussed in Experiment 2, casual observations of our animal subjects, as well as empirical observation suggest differential ability to initiate vs. maintain a social relationship. It would be interesting to examine how much of this altered social competency is purely from the hyperactive symptoms associated with FAE, and how much of the persistence in social interaction initiation is memory and attention based.

Finally, and closely related to alterations in adaptive behavior, we found that ethanol exposure during the brain growth spurt, despite the negative intoxicating effects, led to a dramatic increase in intake during adolescence. Because most long-term drinkers started abusing alcohol during adolescence, the fact that individuals with fetal alcohol exposure preferred to drink alcohol in a free-choice situation suggests that they are at a higher risk for developing substance abuse disorders than the general population. Therapeutic treatments and interventions should therefore track not only the cognitive development of children and adolescents with FAE, but the progress of social development, in order to improve the quality of life as well as to proactively prevent circumstances leading to the onset of drinking behavior.

One purpose of these experiments was to test the efficacy of the postnatal model of binge ethanol exposure to accurately reflect the altered behavioral development in human adolescents with fetal alcohol exposure. We believe that this model provides the strongest evidence of the characteristic behavioral alterations evident in the adolescent expression of fetal alcohol effects in humans and animals.

This is further evidence of the critical importance of timing of alcohol administration in modeling the cognitive and behavioral alterations associated with fetal alcohol exposure in humans. To date, the only consistent behavioral finding from the prenatal ethanol exposure in the rat is hyperactivity. However, administration of ethanol during the period associated with brain growth, a time critical for normal behavioral development, has once again been shown to accurately mimic the behavioral expression of the human condition. One example of the differential effects of pre- vs. postnatal ethanol exposure was Hayne, Hess, and Campbell (1992). When using the prenatal ethanol exposure model of human FAE, Hayne et al. failed to demonstrate any effects of alcohol on the rate of response habituation in pre-weanling animals. Prior work in our lab, in addition to Experiment 1, modified this study by utilizing the postnatal model of ethanol exposure. We demonstrated that ethanol causes robust deficits in response habituation to an odor. Our findings better reflect the expression of FAE in humans as demonstrated by the work of Streissguth et al. (1983), which showed that infants with known alcohol exposure during gestation show altered rates of response habituation.

In addition, our chosen methodology for ethanol administration in the postnatal model seems to be a better choice than the artificial rearing method; especially in light of the finding that postnatal ethanol disrupts the social functioning of adolescent animals. It would be impossible to examine ethanol's effects on social investigatory behavior if the animal was reared in the socially deprived environment of artificial rearing (i.e. the pup-in-a-cup procedure).

Several labs across the country are adopting and furthering the efficacy of the postnatal intubation model of fetal alcohol exposure. The work of Goodlett and colleagues at IUPIU has supplied both behavioral and anatomical insight into the understanding of the manifestation of fetal alcohol effects. Major contributions of this lab include emphasis on the timing and patterns of ethanol administration in developing models for the human condition (Goodlett & Johnson, 1999), as well as evidence that ethanol exposure has a long-lasting impact on spatial learning, and eye-blink conditioning, and anatomical evidence that ethanol damages cerebellar Purkinje cells.

In addition, Kelly and colleagues at the University of South Carolina have used the postnatal model of ethanol delivery to model and investigate ethanol-induced changes in social behavior across the lifespan. The work of this lab has contributed a more global perspective of how fetal alcohol exposure impacts the social and adaptive abilities crucial to everyday life.

Finally, the work of Hannigan and colleagues at Wayne State University has added to the anatomical understanding of fetal alcohol exposure, recently implicating damage to CA1 neurons in the hippocampus. In addition, this lab has also incorporated the examination of ameliorative effects to counter the damage induced by early exposure to ethanol. Currently, they are investigating the therapeutic effects of environmental enrichment, diet, and trophic factors (which promote health in neurons).

The contributions of these labs, in combination with our continued exploration of the alcohol-induced alterations in behavioral expression across the lifespan account

for some of the most exciting research in current developmental psychobiology. By using the postnatal intubation model of ethanol exposure as a standard, we can confidently work towards a unified and comprehensive description of the anatomical and behavioral manifestations of fetal alcohol exposure.

In conclusion, even in the healthiest of cases, the period of transition from childhood to adulthood, known as adolescence, can be particularly stressful. Fetal alcohol exposure can further compound this tumultuous stage in life through altered behavioral development including impaired attention and memory, disrupted social competency, and increased risk for developing substance abuse disorders. The work accomplished by this thesis strengthens the animal model of postnatal ethanol exposure which strongly maps onto the obstacles faced in the human expression of fetal alcohol effects during adolescence.

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Figure 1.

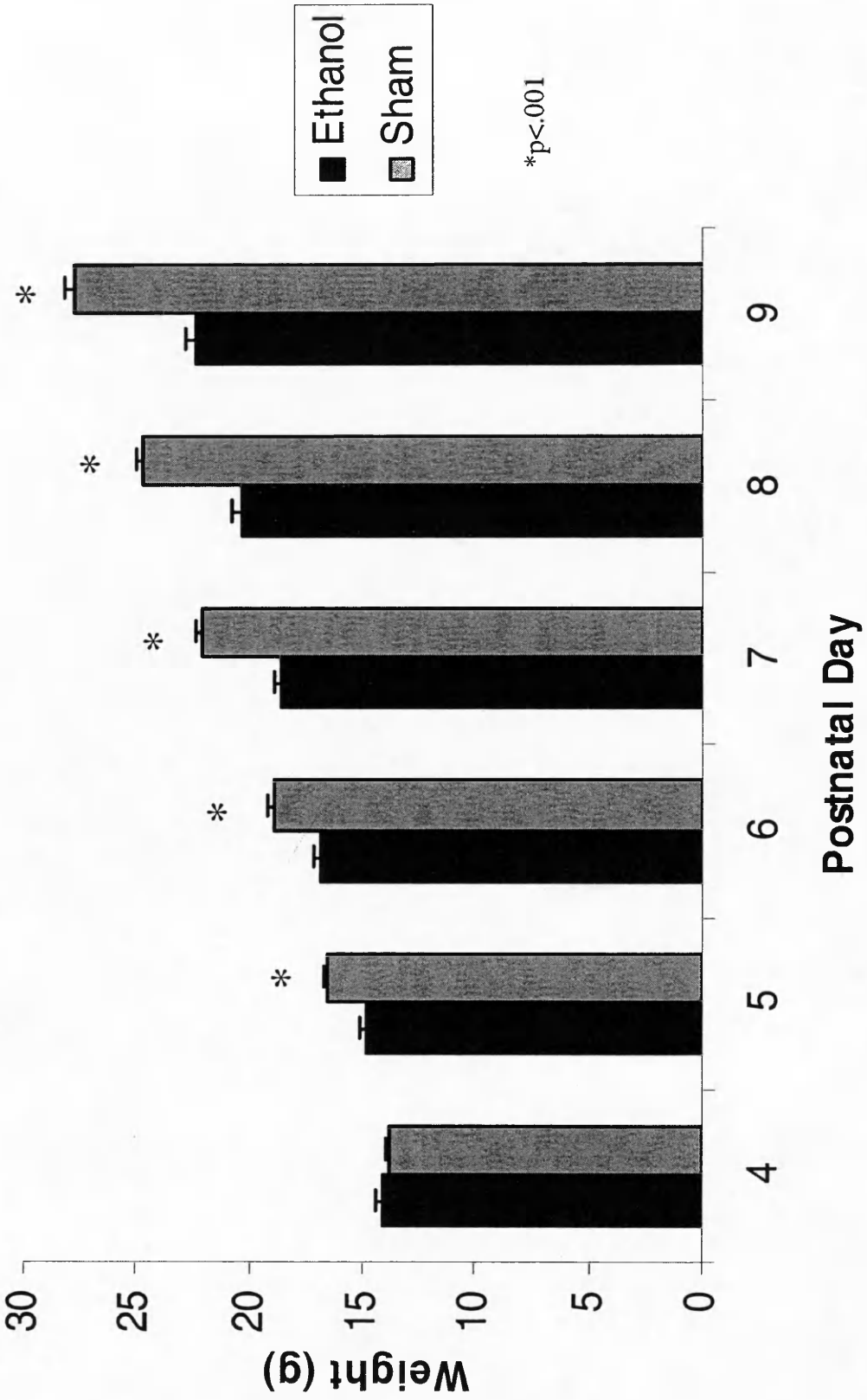

Ethanol
Sham
*p<.001
Weight (g)
0
5
10
15
20
25
30
4
5
6
7
8
9
Postnatal Day
*
*
*
*
*

Table 1.

		Mean Weight (g) *(SEM)*	
Postnatal Day	Sex	Ethanol	Sham
16	Males	37.25 *(2.20)*	41.58 *(2.20)*
	Females	37.60 *(2.04)*	42.28 *(1.99)*
23	Males	66.05 *(3.71)*	71.86 *(4.01)*
	Females	59.83 *(4.91)*	69.11 *(4.01)*
30	Males	109.53 *(6.06)*	111.24 *(5.35)*
	Females	95.35 *(9.26)*	108.96 *(8.02)*

Figure 2.

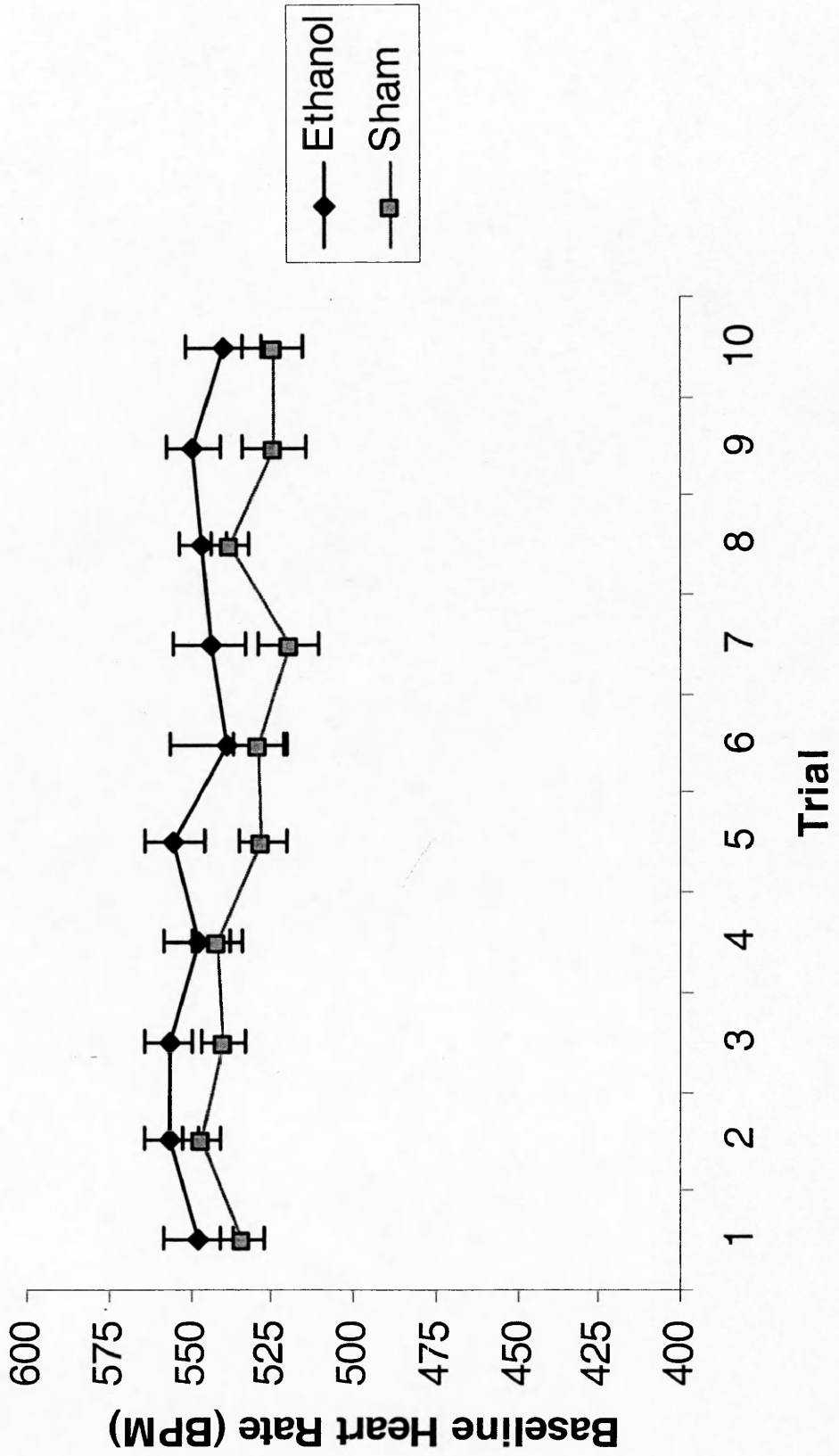

Ethanol
Sham
Baseline Heart Rate (BPM)
600
575
550
525
500
475
450
425
400
1
2
3
4
5
6
7
8
9
10
Trial

Figure 3.

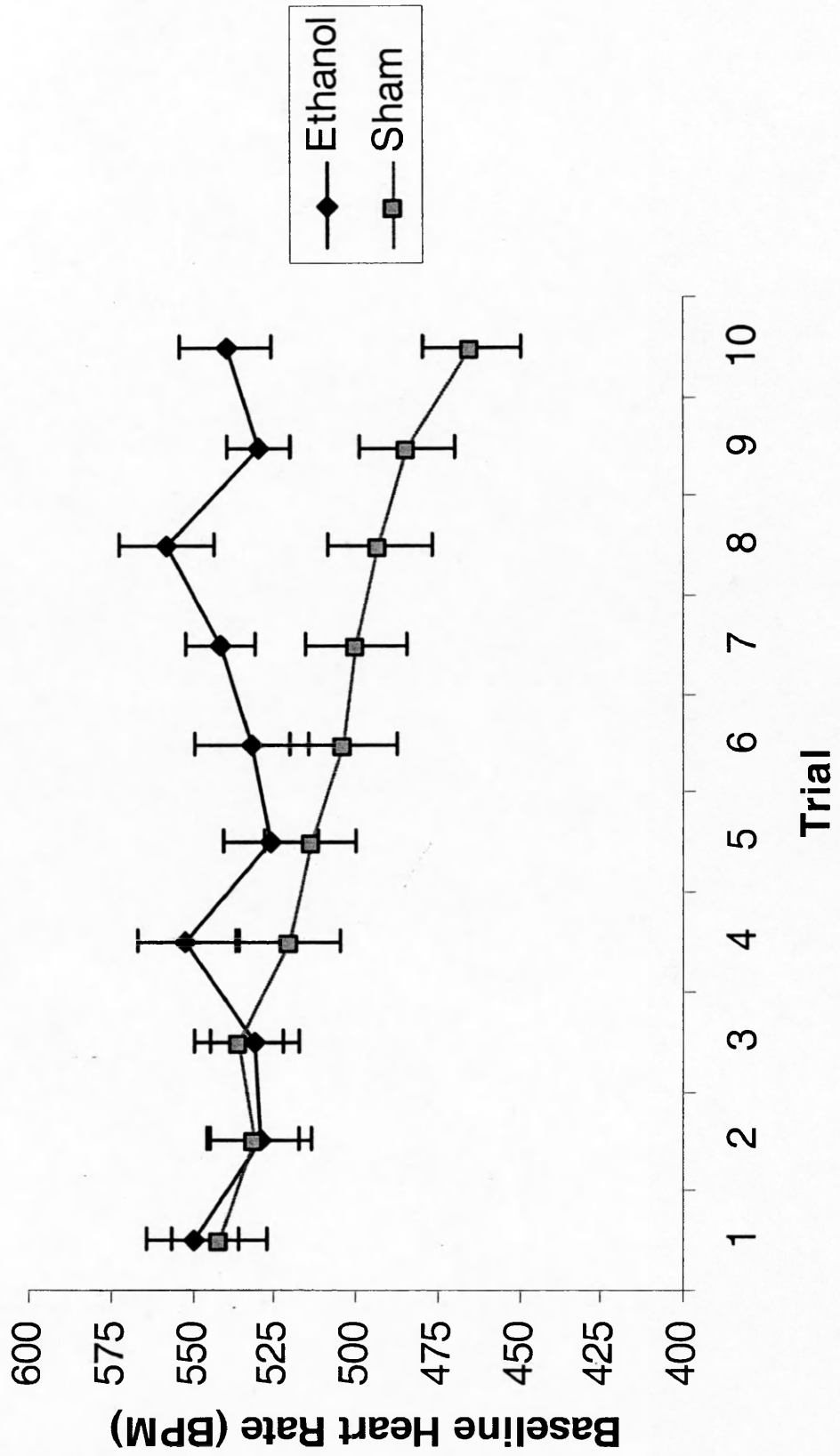

Ethanol
Sham
Baseline Heart Rate (BPM)
600
575
550
525
500
475
450
425
400
1
2
3
4
5
6
7
8
9
10
Trial

Figure 4.

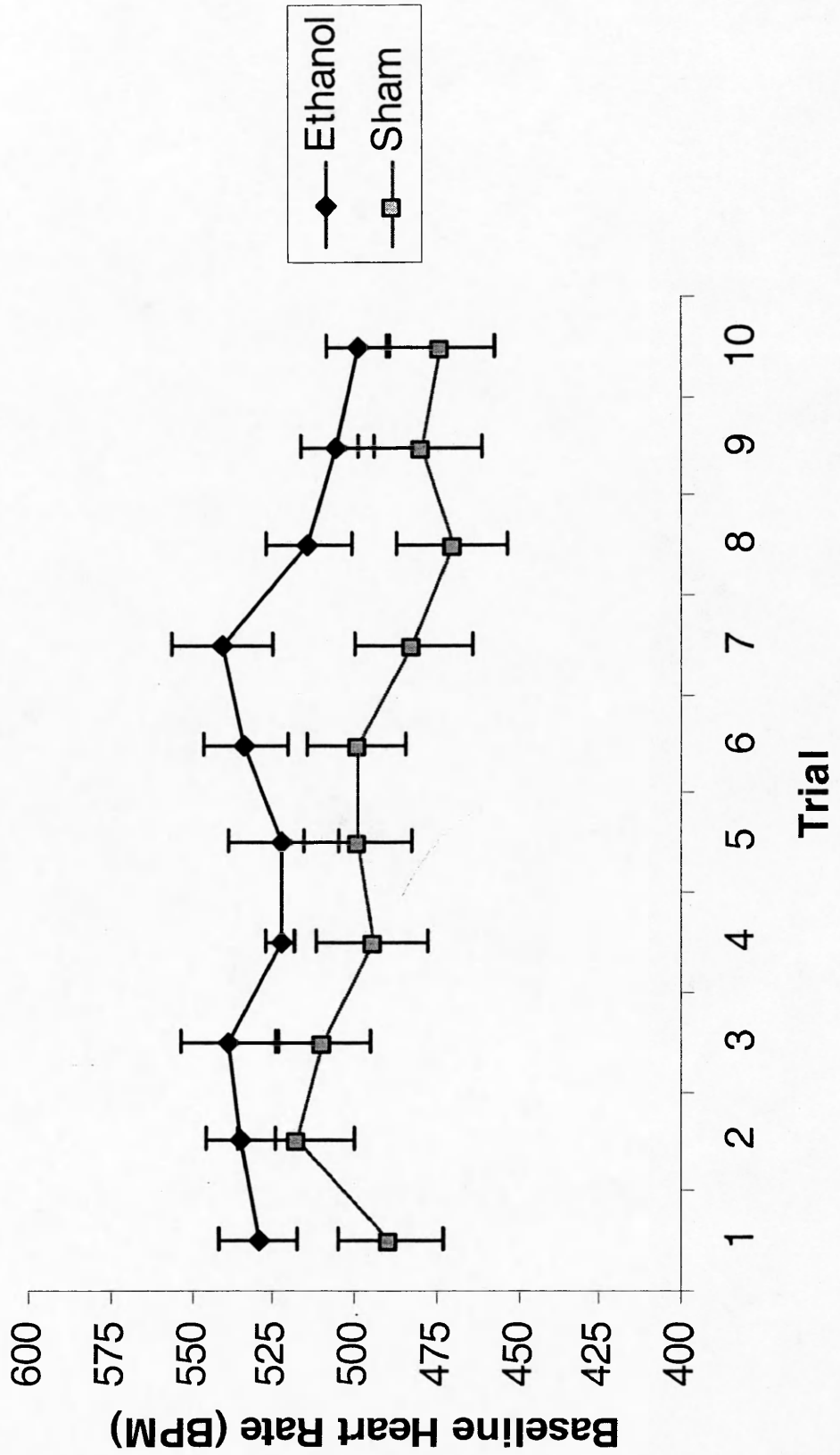

Ethanol
Sham
Baseline Heart Rate (BPM)
600
575
550
525
500
475
450
425
400
1
2
3
4
5
6
7
8
9
10
Trial

Figure 5.

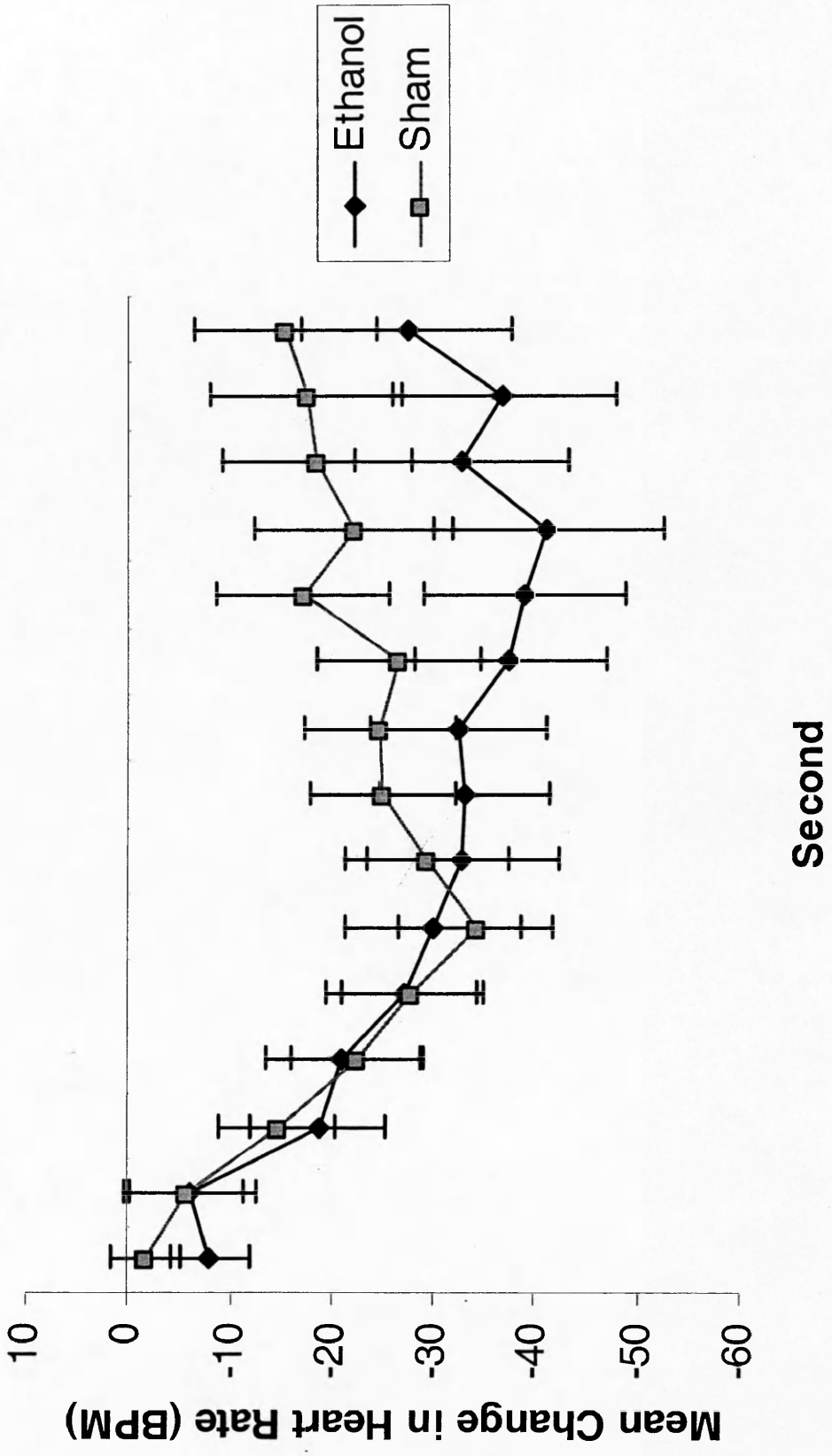

Ethanol
Sham
Second
Mean Change in Heart Rate (BPM)
10
0
-10
-20
-30
-40
-50
-60

Figure 6.

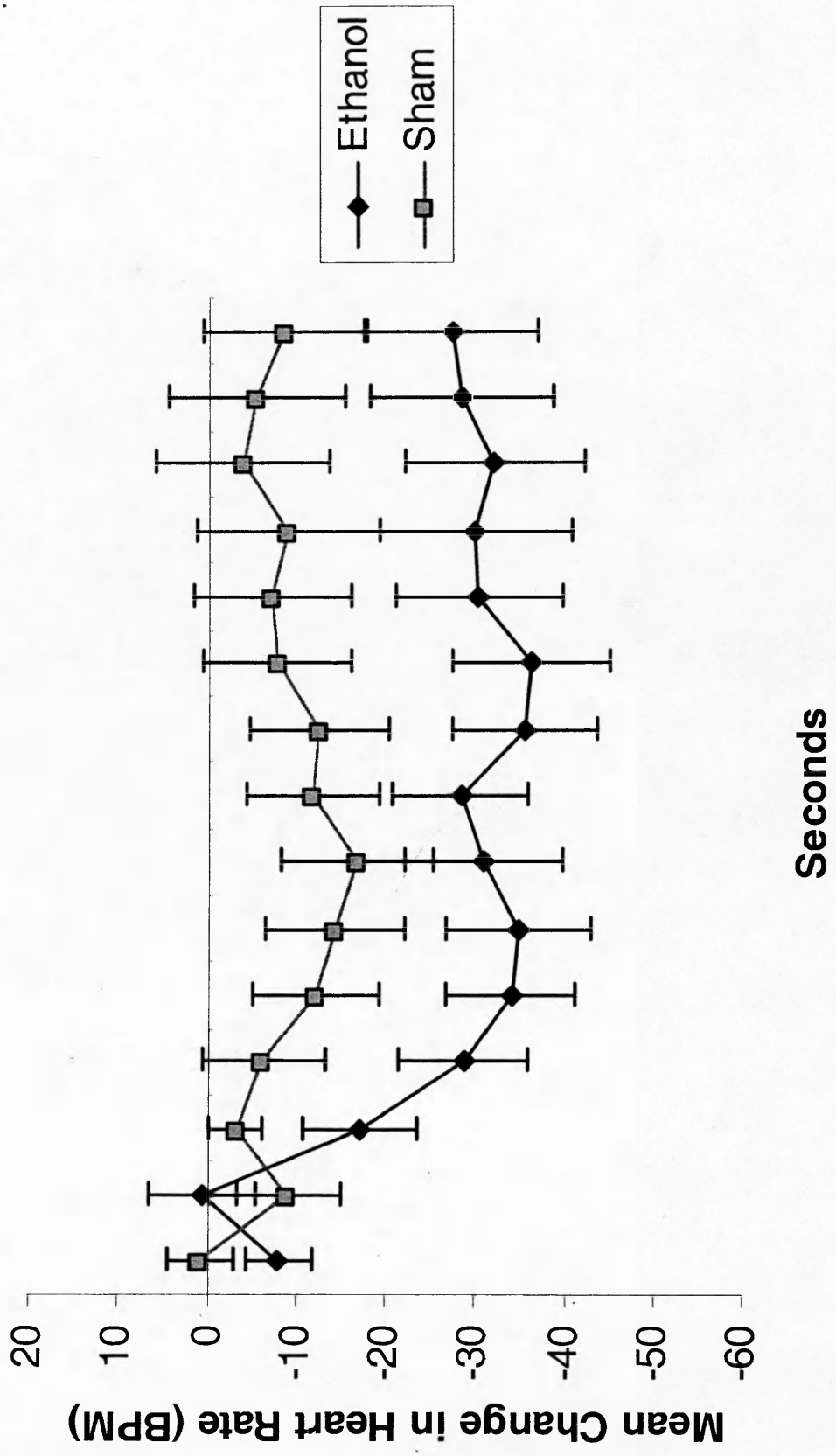

Ethanol
Sham
Mean Change in Heart Rate (BPM)
20
10
0
-10
-20
-30
-40
-50
-60
Seconds

Figure 7.

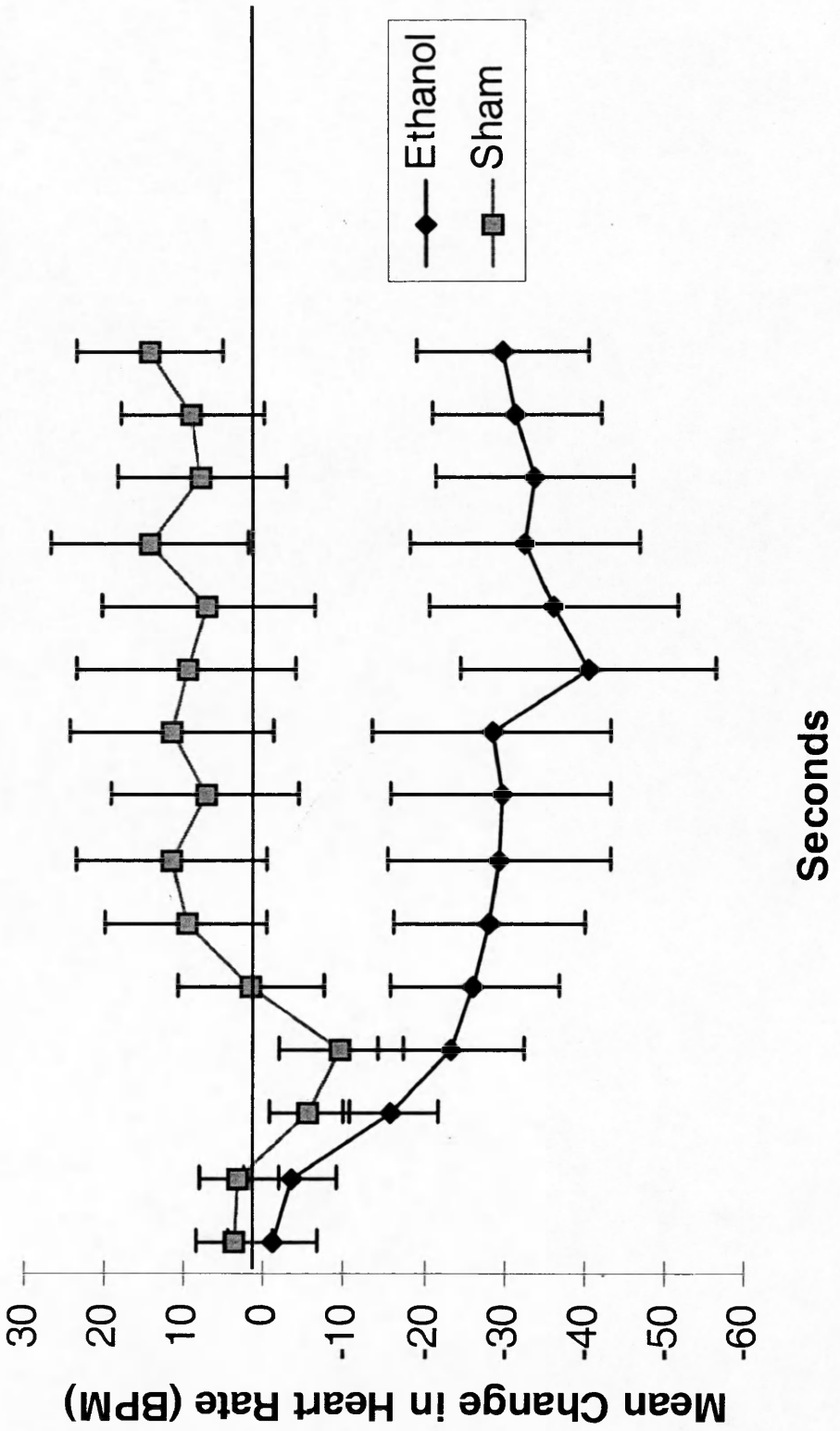

Ethanol
Sham
Mean Change in Heart Rate (BPM)
30
20
10
0
-10
-20
-30
-40
-50
-60
Seconds

Figure 8.

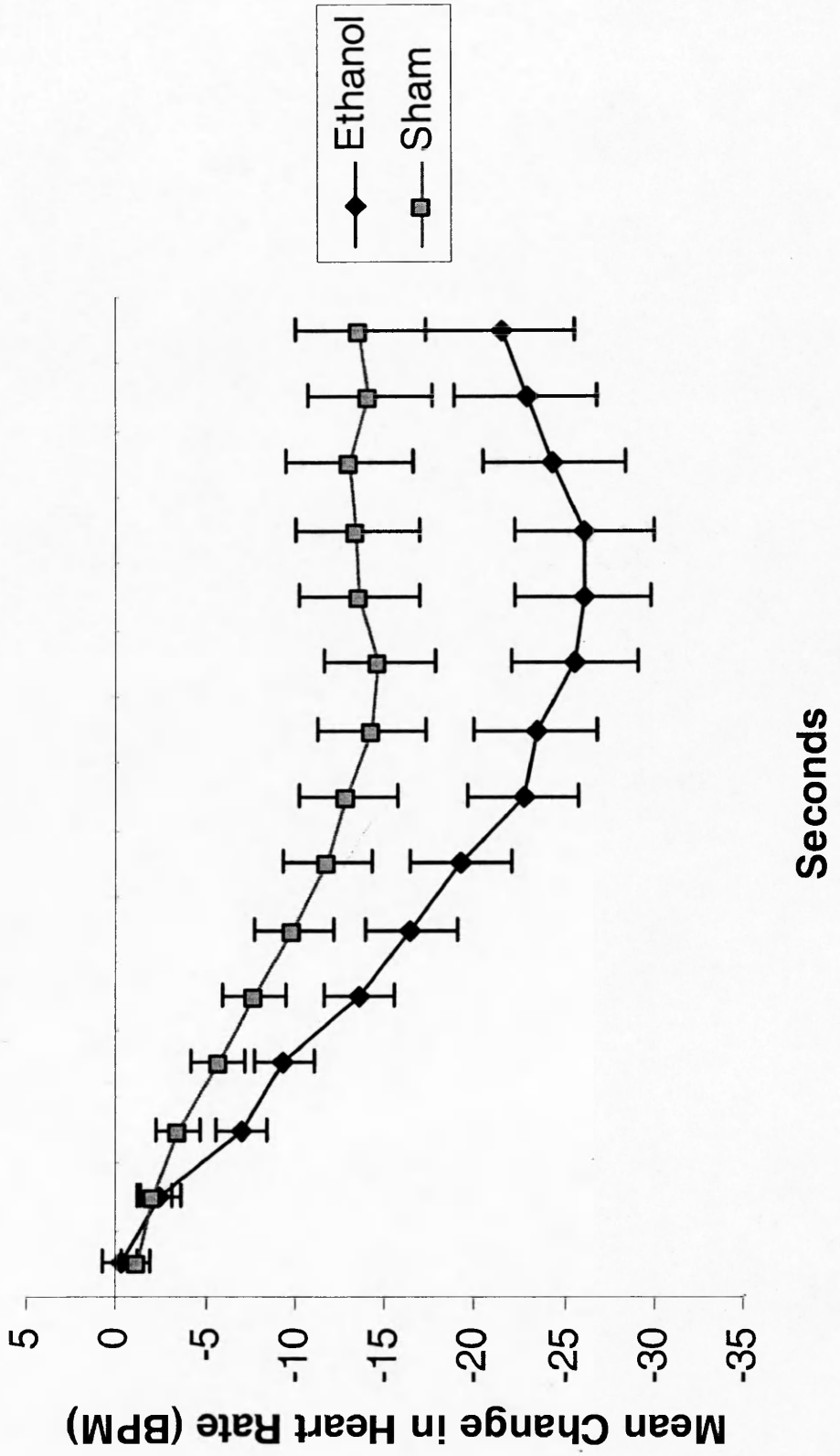

Ethanol
Sham
Mean Change in Heart Rate (BPM)
5
0
-5
-10
-15
-20
-25
-30
-35
Seconds

Figure 9.

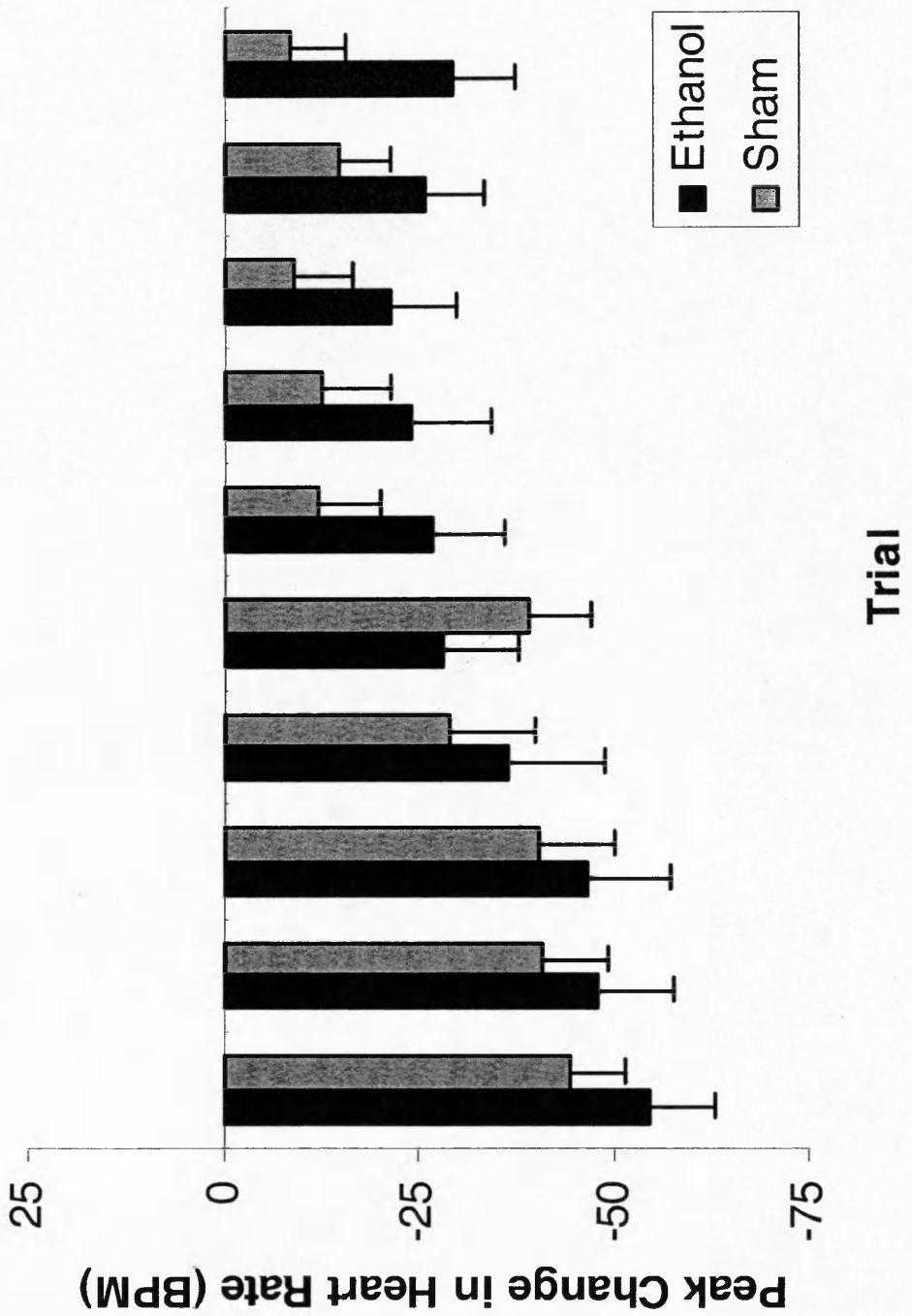


Figure 10.

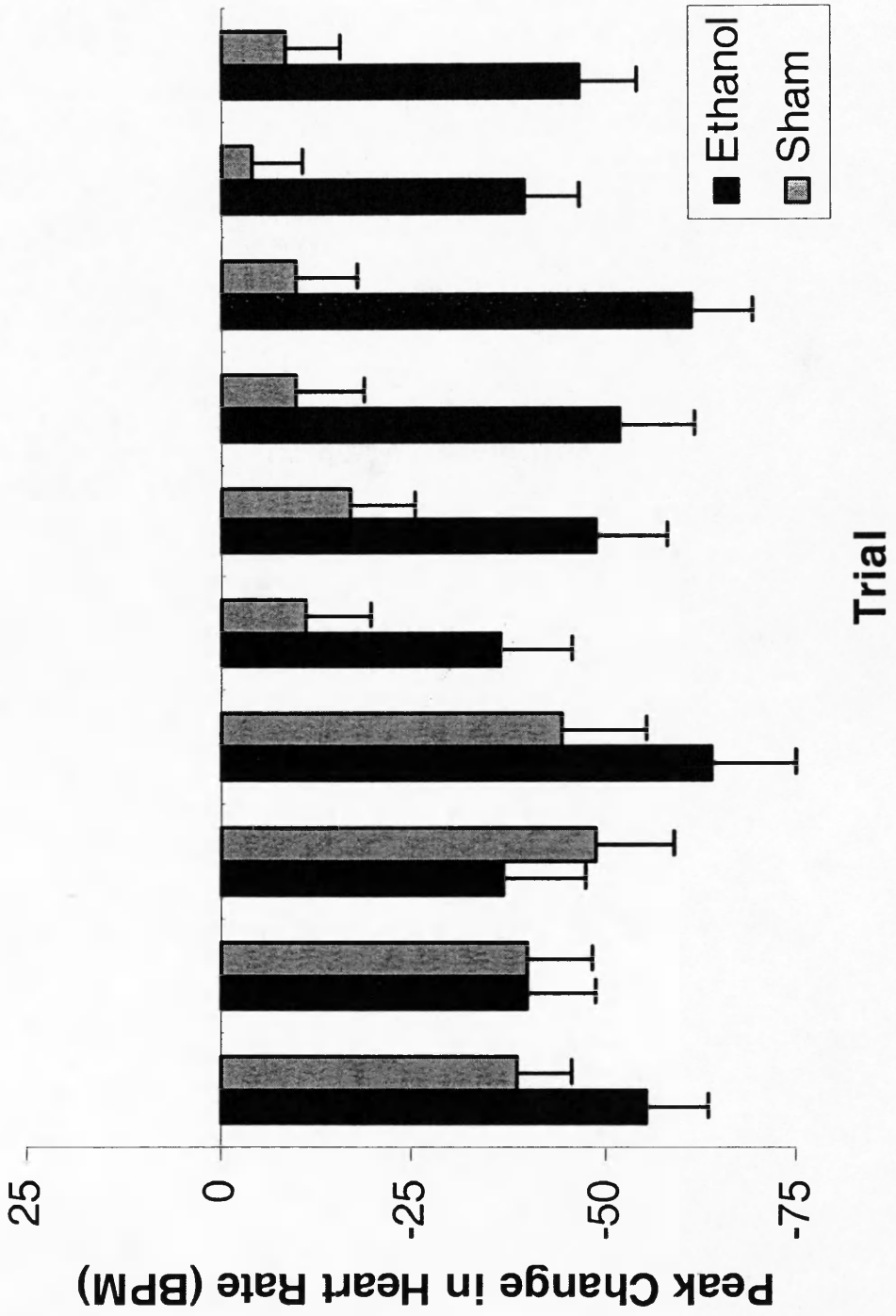


Figure 11.

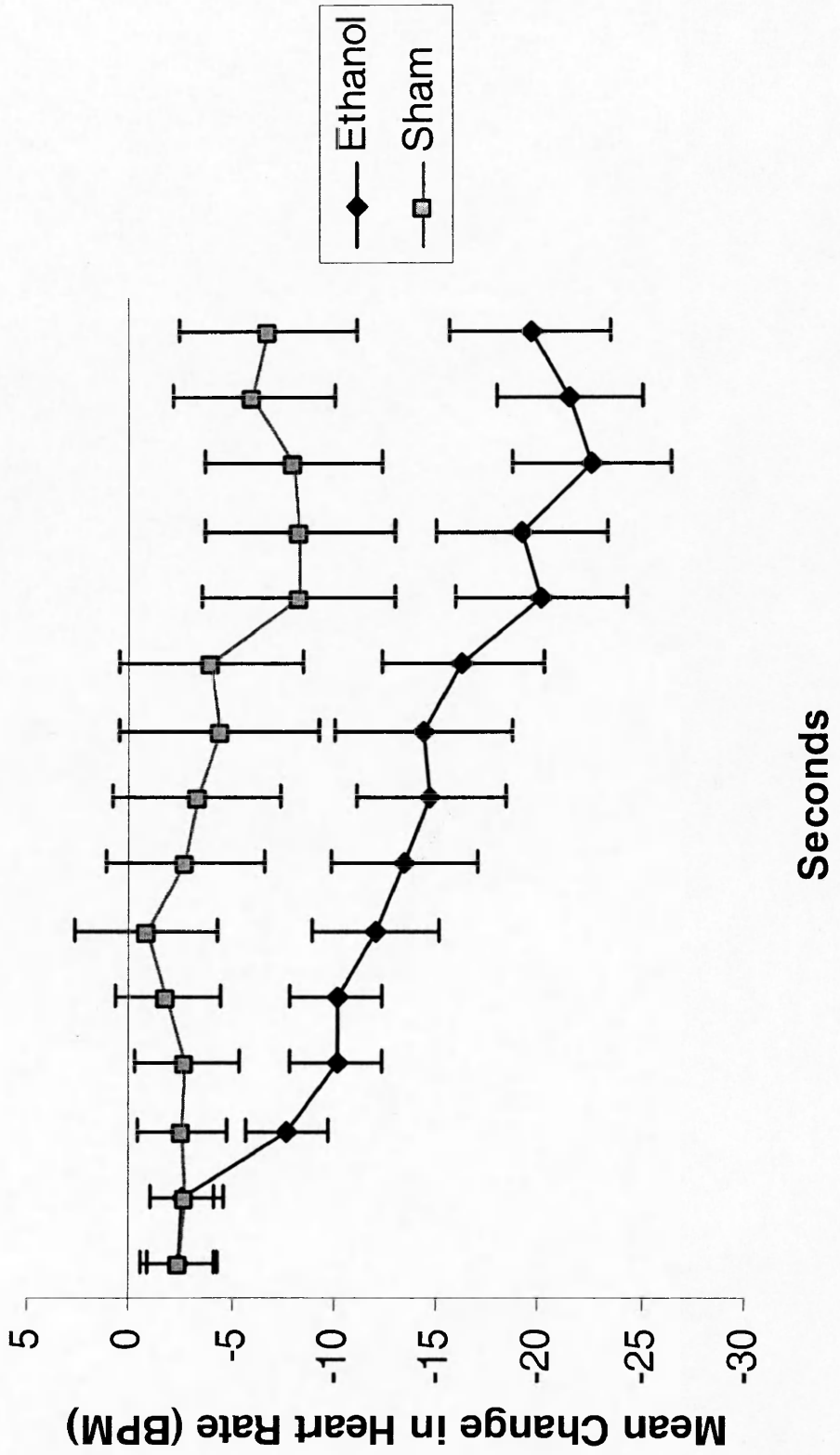

Ethanol
Sham
Mean Change in Heart Rate (BPM)
5
0
-5
-10
-15
-20
-25
-30
Seconds

Figure 12.

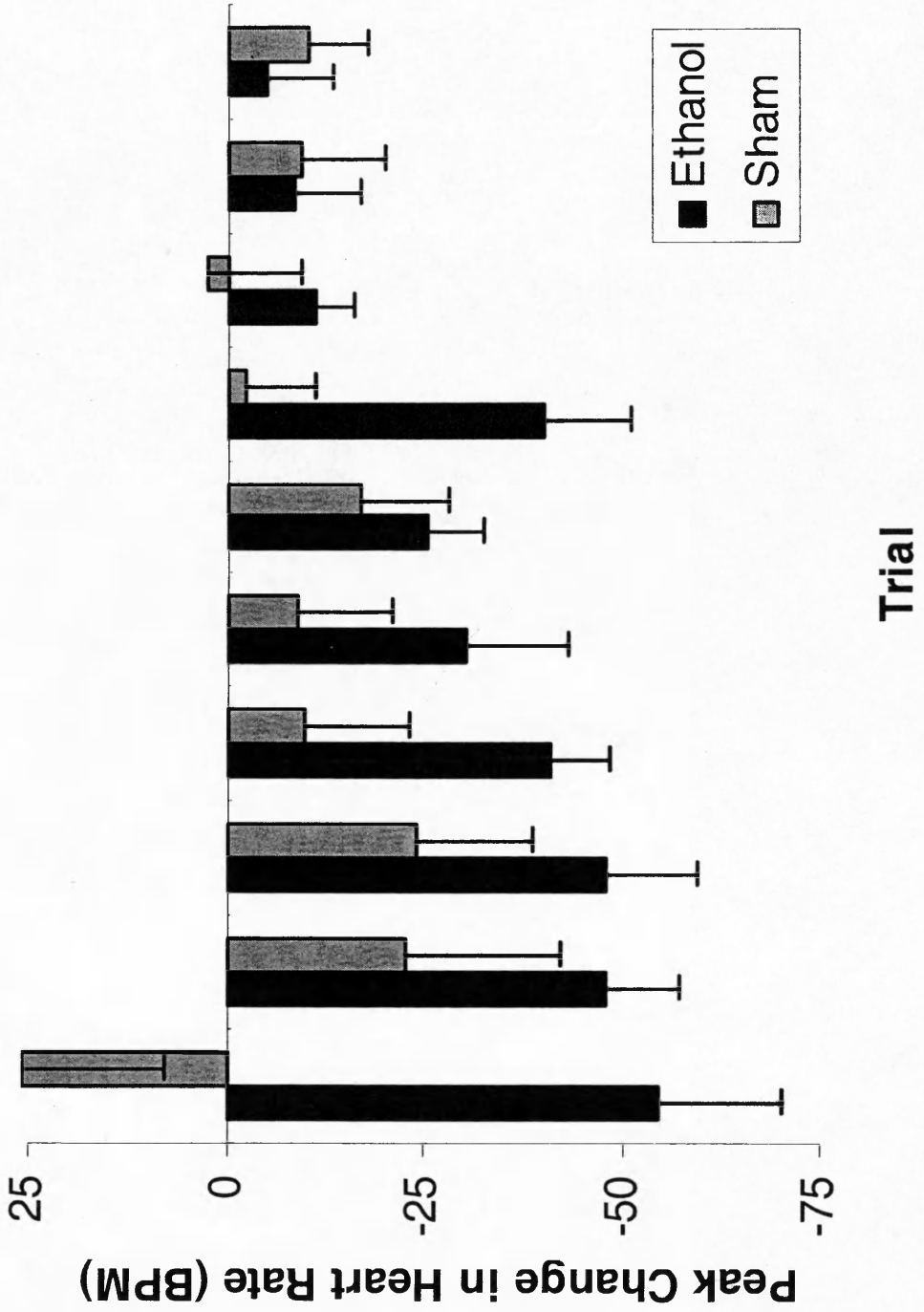


Figure 13.

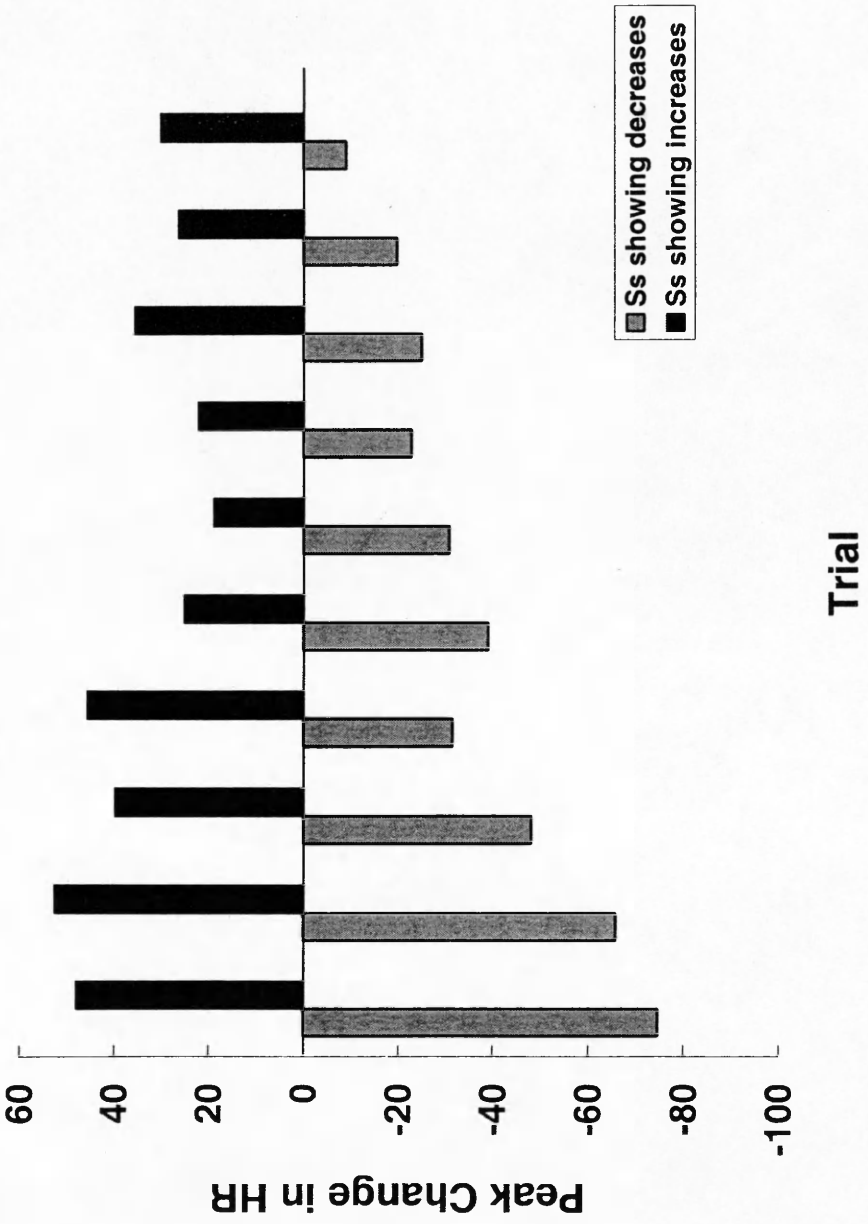

Ss showing decreases
Ss showing increases
Trial
Peak Change in HR
60
40
20
0
-20
-40
-60
-80
-100

Table 2.

		Interval Length (minutes)			
		1	2	4	8
Behavior		Mean Proportional Scores (*SEM*)			
Sniffing	EtOH	0.94 *(.18)*	1.52 *(.28)*	1.18 *(.30)*	1.25 *(.20)*
	Sham	0.78 *(.13)*	1.04 *(.29)*	0.95 *(.10)*	1.17 *(.19)*
Grooming	EtOH	0.34 *(.12)*	1.25 *(.55)*	0.52 *(.19)*	1.18 *(.32)*
	Sham	0.88 *(.24)*	1.47 *(.51)*	0.66 *(.15)*	1.20 *(.18)*
Boxing	EtOH	0.46 *(.15)*	0.28 *(.07)*	0.92 *(.25)*	0.65 *(.18)*
	Sham	0.55 *(.24)*	0.55 *(.18)*	0.55 *(.14)*	0.84 *(.20)*
Following	EtOH	0.51 *(.06)*	0.51 *(.08)*	0.78 *(.20)*	0.91 *(.13)*
	Sham	0.38 *(.09)*	0.49 *(.10)*	0.60 *(.09)*	.067 *(.09)*
Pinning	EtOH	0.21 *(.09)*	0.43 *(.17)*	0.89 *(.17)*	1.00 *(.19)*
	Sham	0.43 *(.12)*	0.28 *(.06)*	0.41 *(.13)*	0.43 *(.14)*

Figure 14.

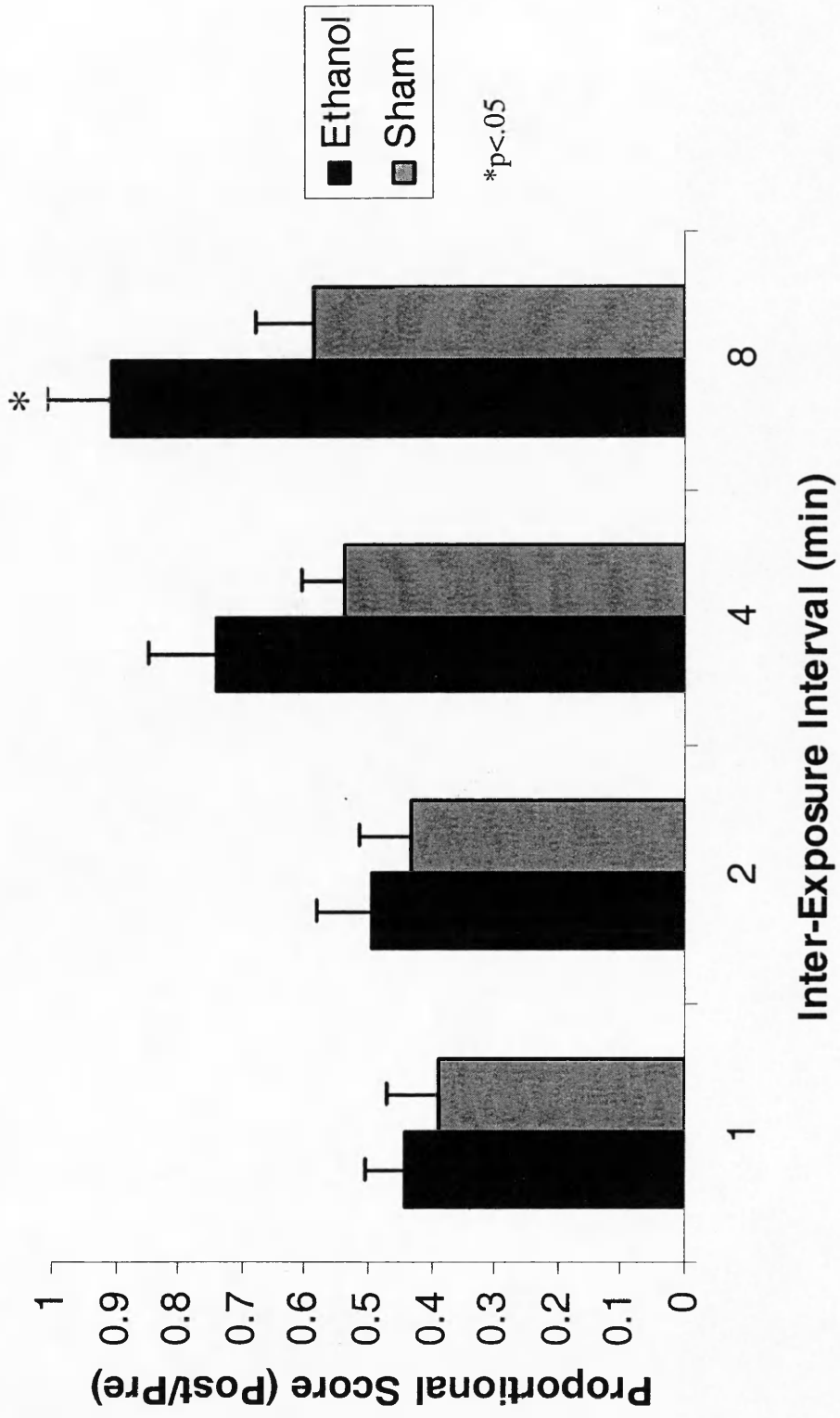
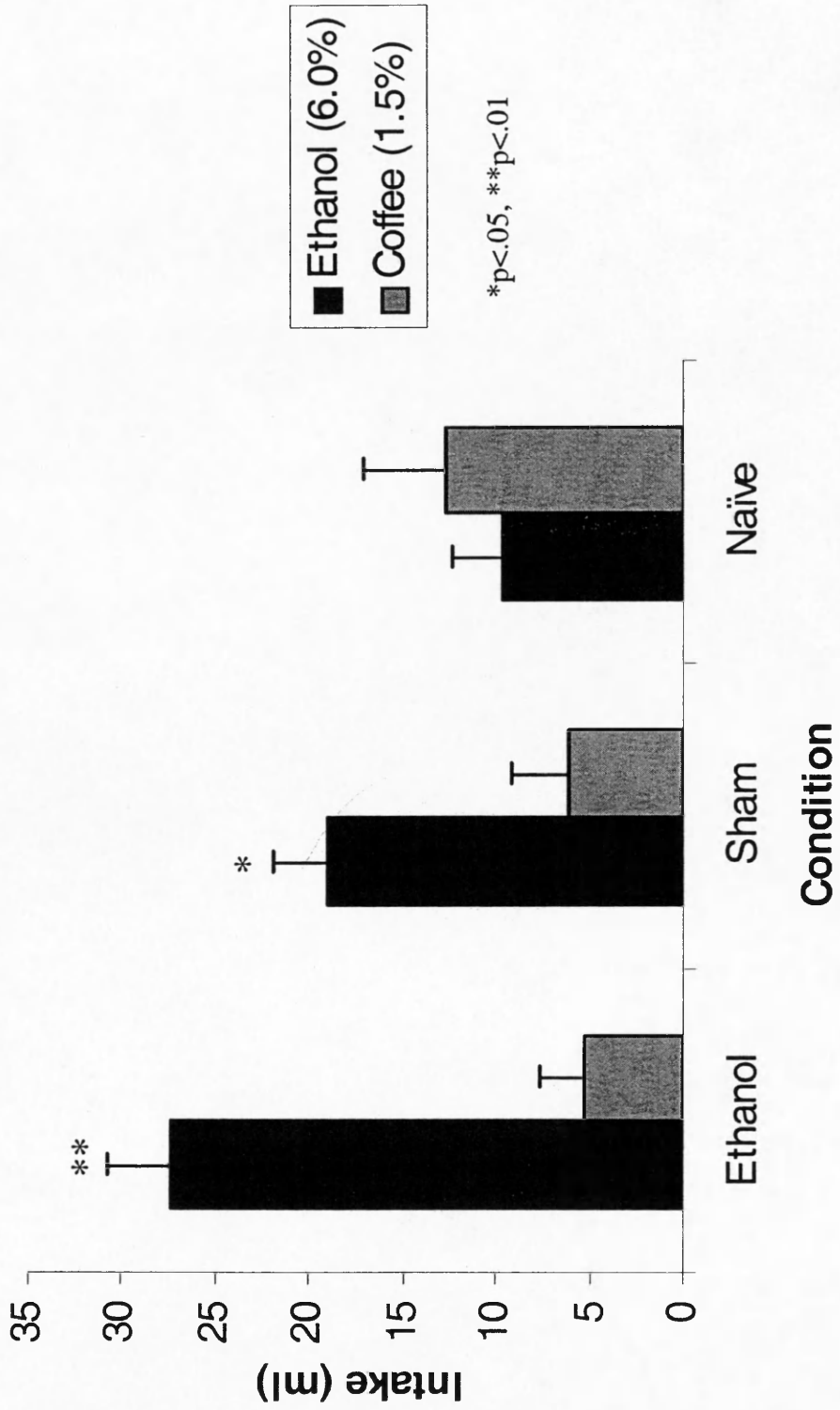

Ethanol
Sham
*p<.05
Proportional Score (Post/Pre)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1
2
4
8
Inter-Exposure Interval (min)
*

Figure 15.


Ethanol (6.0%)
Coffee (1.5%)
*p<.05, **p<.01
Intake (ml)
0
5
10
15
20
25
30
35
**
*
Ethanol
Sham
Naïve
Condition

VITA

Katherine Anne Colona

Katherine Anne Colona completed her education in Burlington, New Jersey, graduating as Valedictorian of Burlington City High School in 1997. Following her graduation, she matriculated to the College of William & Mary in the fall of that year.

During her undergraduate years at the College of William & Mary, Katherine received a B.S. in Biological Psychology, under the advisement of Dr. Pamela Hunt. Her independent study in Dr. Hunt's laboratory fostered a strong love of research, as well as great interest in psychology.

During her time as a Masters student at The College of William and Mary, Katherine continued to work closely with Dr. Hunt, as well as Dr. Robert Lennartz, Dr. Adam Rubenstein, and Dr. Josh Burk, who helped to refine her interests in developmental psychobiology and behavioral neuroscience.

Katherine will enter the Developmental Cognitive Neuroscience doctoral program at Virginia State and Polytechnic University, in Blacksburg, Virginia in August 2003.